Immunofluorescence in Renal Disease

A clinicopathological study of 300 consecutive renal biopsies

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Submitted for the degree of

Doctor of Medicine

to the Faculty of Medicine

University of Edinburgh

1978

TO MARION, ANDREW

PAMELA AND IAIN

They also serve who only stand and wait.

Milton

A kidney can always suffer the loss

of a millimeter of substance.

Gwyn 1923

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I INTRODUCTION

The kidney may be involved in many pathological processes. In some instances the involvement is as part of a widespread systemic disease whereas in other cases the renal disease is the prime pathology which may or may not be associated with systemic complications. This has caused considerable difficulty with classification as clinical manifestations of renal disease are relatively limited. Proteinuria, haematuria, hypertension and diminished renal function are the hallmarks of renal pathology and although patients may have many other symptoms and signs these are usually secondary consequences.

The clinical syndromes associated with glomerular diseases have been described by various authors over the past 150 years. Interest in glomerular disease has expanded out of all recognition over these years and now involves not only clinicians but immunologists, pathologists, microbiologists, epidemiologists and immunochemists. Although understanding of glomerulonephritis has increased the words of Richard Bright are now more than ever applicable:

> "it may be fairly asserted that no other disease is more interesting in its history and origin, affords so many instances of the consent and dependence of the different functions upon each other, gives so much scope for pathological observations, or is so fertile in facts interesting to the cultivation of animal chemistry as the disease of which we now speak."

Bright (1827) established the connection between diseased kidneys, proteinuria and oedema. Since then there have been many attempts to correlate the clinical findings with the pathological appearance of post-mortem or renal biopsy material. Classical descriptions, which still form the basis of modern terminology, were documented by Volhard and Fahr (1914) although the separation of acute nephritis from nephrotic syndrome was emphasised more by Longcope (1936) and subsequently by Ellis (1942). It is interesting to note that the remarks of Ellis (1942) are still correct 35 years later:

> "Much of the controversy which still exists in our knowledge of the conditions grouped under the name of Bright's disease arose from failure to study the natural history of these conditions - their mode of incidence and subsequent course to death or recovery."

The increased understanding of glomerulonephritis in recent years has been due to three major developments : the development and detailed study of experimental models, the introduction and widespread use of renal biopsy, and the development of investigative techniques such as immunofluorescence microscopy.

The involvement of immunological processes in glomerulonephritis was suggested by Schick (1907) who compared the latent period between streptococcal infection and acute nephritis to the delay in the appearance of serum sickness after heterologous serum injections. Since then there have been many studies of the effect of the injection of antigen in animals. Hawn and Janeway (1947) demonstrated a diffuse proliferative glomerulonephritis after single injections of either bovine serum albumin or bovine gamma globulin in rabbits.

It was thought that the lesions were due to antigen-antibody reactions in the tissue where antigen had been localised as the acute lesions were present only when antigen was present and before antibody appeared in the circulation. Schwab and his co-workers (1950) showed that there was a fall in serum complement at the time of disappearance of antigen and before the appearance of antibody, and that complement then rises as antibody appears in the circulation. These findings suggested that antibody formation was necessary for the development of glomerulonephritis. Germuth (1953) suggested that the lesions could be due to soluble antigen-antibody complexes rather than antibody reaction with fixed antigen. The deposition of gamma globulin in the glomerulus during experimental serum sickness was shown by Mellors et. al. (1955) using immuno-fluorescent techniques. Further studies (Dixon et. al. 1958) demonstrated that the antibody was not localised in the glomerulus until the period of immune elimination of antigen. It is concluded from these studies that experimental glomerular lesions are due to the deposition of circulating soluble antigen-antibody complexes and the reactions which follow. These studies explain the development of acute nephritis following a streptococcal infection but in many patients there is no clear clinical pattern of bacterial infection, latent period and acute nephritis.

Two experimental models which may explain the apparent lack of acute infection prior to the development of acute nephritis are the spontaneous nephritis of mice infected with lymphochoriomeningitis virus and the nephritis of New Zealand black mice. In mice infected with lymphochoriomeningitis (LCM) virus there is the steady

accumulation of LCM antibody, complement and viral antigen in the glomerulus of affected animals with the subsequent development of glomerulonephritis. Similarly in New Zealand black mice there is deposition of antinuclear antibodies, complement and nuclear antigens in the glomerulus. These models are similar to lupus erythematosus and the nephritis associated with persistent infection such as quartan malaria. It is likely that in these conditions there is immune complex formation with subsequent glomerular localisation and the development of glomerulonephritis. From these studies it appears that some types of human glomerulonephritis are associated with glomerular deposition of immune complexes following antigen exposure and antibody formation.

A second experimental model is that of antikidney antibody. In this model homogenised renal cortex is injected into an animal and after antibody has been produced serum is obtained and injected into another species. This is followed by the abrupt onset of proteinuria, haematuria and oliguria. This model has been termed Masugi nephritis after the early studies of Masugi (1933). The nephrotoxic serum nephritis exists as two phases : an immediate phase where heterologous antikidney antibody is demonstrable on the glomerular basement membrane, and a second stage in which homologous gamma globulin is deposited in the glomerulus after a latent period. The consequences of the injection of heterologous antikidney antibody is dependent on the dose of antibody injected, the species in which it was produced and the state of the immune system in the injected animal. Nonetheless the experimental disease is in many ways similar to Goodpasture's Syndrome when circulating anti-glomerular basement membrane antibodies

can be identified.

There are thus two main mechanisms of immune glomerular injury : the first may be attributed to circulating soluble immune complexes which become deposited in the glomerulus, and the second due to antibody directed against the basement membrane of the glomerulus. From many studies of human renal biopsies it would appear as though the immune-complex mediated is by far the most common whereas that induced by antikidney antibody is rare (Berger et. al. 1971).

A further significant advance in the understanding of renal disease, particularly with respect to glomerular disease, occurred with the development of the technique of percutaneous renal biopsy. This technique allowed histological examination of tissue to be undertaken, sometimes repetitively, with minimal inconvenience to the patient. Perez Ara (1950) reported that percutaneous biopsy was a safe and relatively simple clinical procedure and this was soon confirmed by Iversen and Brun (1951). Since then there have been numerous reports on the value of this technique in the diagnosis and management of patients with glomerular disease. The complications which may occur following renal biopsy are few, if proper post-biopsy care is followed, but include haemorrhage, either into the urinary tract or perirenal, infection and arteriovenous fistulae. Haemorrhage tends to occur most often in hypertensive patients and those with vascular disease such as polyarteritis nodosa. Care must be exercised in the selection of patients for biopsy and it is generally considered unwise to perform biopsy on a patient with a solitary kidney or in those with bleeding disorders. In such patients, if histological diagnosis is considered essential, it is probably preferable to obtain

tissue by surgical procedure. To put the risks into perspective it would be reasonable to state that haemorrhage requiring transfusion probably occurs in about 1 in 300 biopsies and surgical intervention is required in about 1 in 3000 biopsies.

There are several needles available for obtaining renal tissue, The most common is probably the Franklin modification of the Vim-Silverman needle. This device is a needle and stylet which is inserted in the renal tissue. Once in situ the stylet is removed and replaced by cutting prongs, which are then advanced quickly into the kidney. The outer sheath is advanced over the prongs and the needle and tissue removed. A biopsy obtained in this way will usually measure 1 to 15 mm in length and 2 mm in diameter. Recently a disposable needle has become available (Tru Cut, Travenol Ltd.) in which the stylet is modified so as to obviate the need to insert cutting prongs through the outer sheath, being thus simpler to use. The size of biopsy obtained is much the same as that with the modified Vim-Silverman needle. Renal cortex in the biopsy is essential for the diagnosis of glomerular disease. It is generally considered desirable to have five to ten glomeruli in the light microscopy part of the biopsy but this will depend to a large extent upon the nature of the underlying disease. Occasionally it will be possible to make a diagnosis on a single glomerulus and rarely from the presence of a blood vessel, as in amyloidosis or polyarteritis nodosa.

The technique of immunofluorescence is dependent upon early work which showed that an antibody molecule could be covalently linked with other molecules without disturbing its specific reactivity. Reiner (1930) coupled diazotized atoxyl to antipneumococcal antibodies

which subsequently agglutinated specific pneumococci giving them a brown colour. It was therefore possible to detect an antigen by an antibody which had been labelled to make it visible. Thus the presence of an antigen could be inferred from the localisation of a chemical dye. These early studies were confirmed by Marrack (1934) who coupled a red dye to antibody providing a method of identifying specific bacteria in suspension.

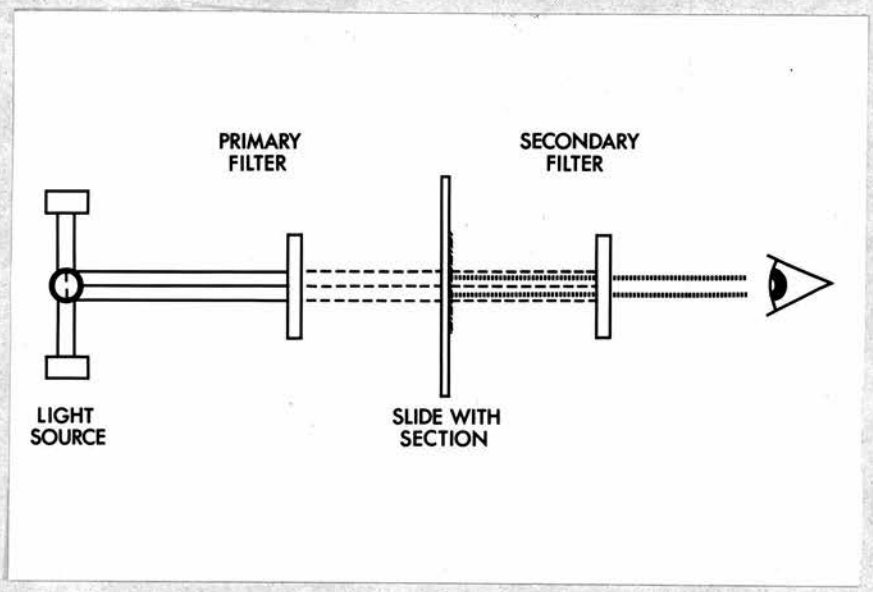
The demonstration of antigen in tissues was first described by Coons et. al. (1942). He coupled antipneumococcal antibody with fluorescein isocyanate and demonstrated free antigen and pneumococci in the Kupffer cells of mice dying of pneumococcal infection. The isocyanate was relatively unstable and before the fluorescence technique could be applied on a large scale a more stable material had to be developed. Riggs et. al. (1958) introduced fluorescein isothiocyanate which being stable opened the way to the more widespread application of immunofluorescence.

In fluorescence microscopy an object stained with fluorescent dye, if suitably excited, emits light so that when viewed against a dark background its presence can be detected. The sensitivity of detection is some 10^2 times that of an ordinary histochemical stain. Fluorescein, the most widely used fluorescent dye, absorbs ultra-violet, violet and blue light and emits green and yellow light. The maximum absorption is at a wavelength of 490 nm and the maximum emission is at 520 nm. Thus if there is a light filter between the light source and the specimen (primary filter) allowing the transmission of light up to approximately 500 nm the fluorescein will be excited and thus emit light at 520 nm. If a second filter

(secondary filter) is placed between the objective and the eyepiece it is possible to only allow transmission of light of a wavelength greater than 500 nm. In this way the exciting light (less than 500 nm) will not be visible whereas the emitted light (at approximately 520 nm) will be visible (Fig. 1). In such a system fluorescein will be seen as a yellow-green colour against a dark background.

There have been many improvements on the originally described microscopy. Initially the microscope used as a light source a high pressure mercury vapour lamp and a transmitted light system. The high pressure lamp had a limited life and was prone to explode. In addition the wavelength characteristic of its emission tended to alter with use. For these reasons the recently introduced filament lamp has many advantages. The transmitted light system was satisfactory providing the fluorescent dye was present in large amounts but was less satisfactory in situations where the dye was only present in small amounts. The resolution was greatly enhanced by the introduction of incident light illumination (Ploem 1967). In this system the exciting light is reflected by a dichroic mirror onto the section. The emitted light is then observed reflected from the surface of the section being examined, (Fig. 2). In this system the exciting light does not have to pass through condencer, glass slide and section before stimulating the fluorescein to emit yellow-green light. In addition the background is not illuminated as much and is therefore darker giving greater contrast. The present day techniques of immunofluorescence thus allows the detection of small amounts of antigen in suitably prepared tissue sections. (Fig 3)

Figure 1



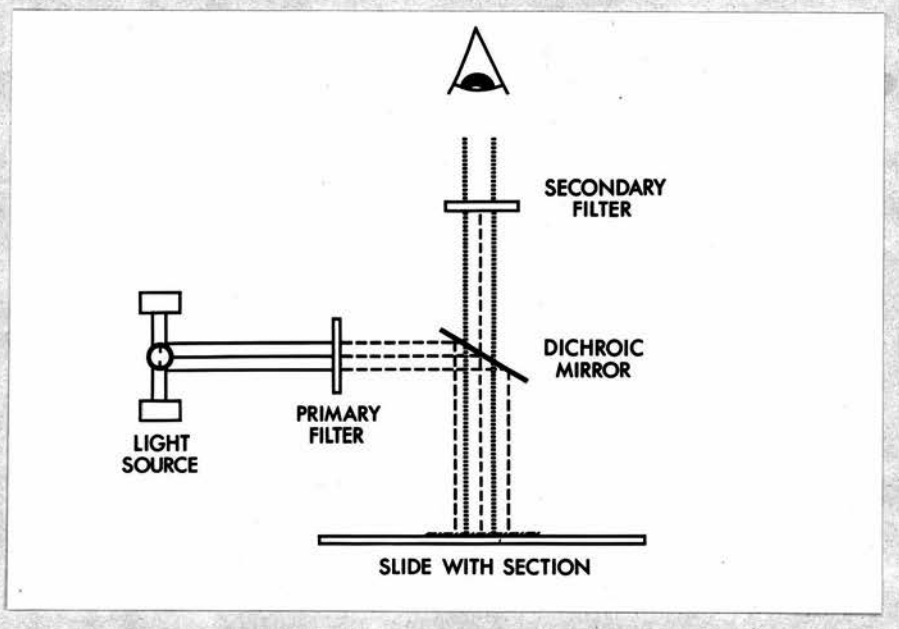
The light source emits light and the primary filter blocks transmission of all except that of wavelength below 500 nm. This light excites fluorescein which thus emits light with a wavelength of 520 nm. The secondary filter allows the transmission of light greater than 500 nm and so only the light emitted from fluorescein excitation is transmitted and observed.

Light from mercury lamp ——————

Wavelength up to 500 nm — — — — — —

Fluorescein emitted light ▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪

Figure 2



In incident light microscopy the exciting light does not have to pass through the glass slide before exciting the fluorescein.

A major problem which remains with this technique is antibody specificity. The results obtained will only be as good as the antibody used. A "clean" antibody should be specific and have no cross-reactions with any other material. There is considerable variation between antiserum obtained from different commercial sources. This is in part due to differences in the antigen used to stimulate antibody production but it is also due to the fact that different species vary in their ability to produce antibodies. For this reason it is frequently difficult to compare the immunofluorescence results obtained in one centre with those of another. For the same reasons it is always important to state the source of antibody when describing results. Fortunately with better antibody raising techniques and greater experience this difficulty is becoming less of a problem.

Following the introduction of satisfactory fluorescence microscopy and fluorescein isothiacyanate conjugates the technique of immunofluorescence microscopy was soon applied to patients with glomerular disease (Mellors and Ortega 1956). The deposition of IgG, IgM and component was demonstrated in the glomerular capillary walls of adults with various types of glomerulonephritis and lupus erythematosus (Koffler and Paranetto 1965). Similar studies in children (Kobayashi 1966) showed the deposition of immunoglobulins and complement in patients with glomerulonephritis and Henoch-Schonlein purpura. These early studies were followed by a number of reports from various centres documenting their results (McCluskey et. al. 1966, Lange et. al. 1966, Hadley 1967, Brentjens et. al. 1969) and since then there have been many further studies. In general the immunofluorescence

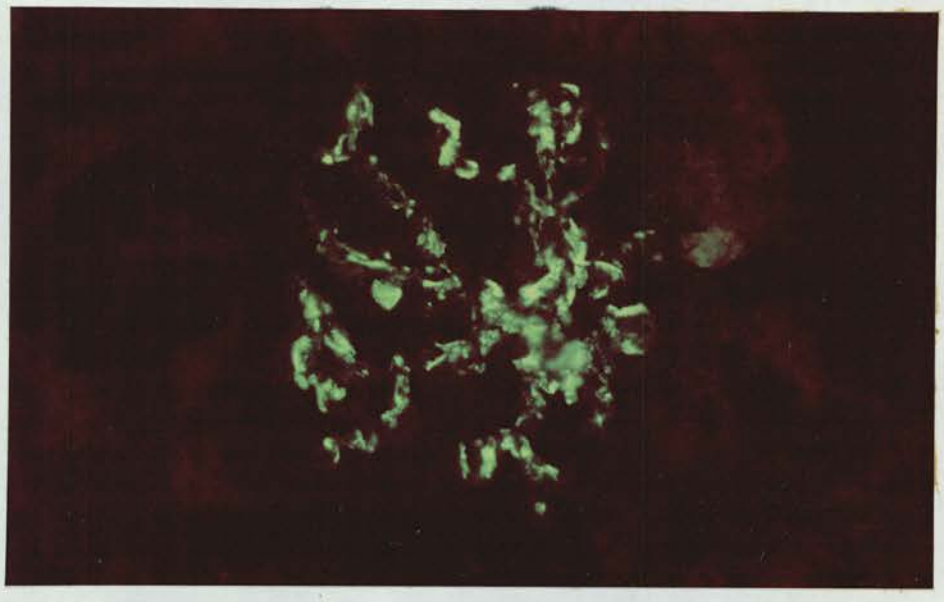
findings support the concept that in most instances glomerulonephritis is an immunologically mediated disease. In addition certain forms of glomerulonephritis have characteristic immunohistological appearances as in membranous and mesangiocapillary glomerulonephritis. An interesting finding is the lack of immunofluorescence in biopsies of patients with minimal lesion glomerulonephritis in spite of its excellent response to immunosuppressive therapy. In spite of the many immunofluorescence studies there remain many unanswered questions regarding glomerulonephritis.

Experimental studies and examination of human renal disease have confirmed the role of immune complexes in the pathogenesis of glomerulonephritis but there is a surprising lack of information regarding the antigens involved. In a few instances, such as Systemic Lupus Erythematosus, malarial nephropathy, post-streptococcal glomerulonephritis and malignancy associated nephrotic syndrome, the antigen is known but these conditions form only a very small proportion of patients with glomerulonephritis. The natural history and the influence of age, sex and therapy, is largely unknown. Clearly more studies are required so that more satisfactory methods of prevention and treatment may be based on more accurate knowledge of the pathogenesis of this heterogenous group of diseases.

The present study was undertaken to examine the relationships between the clinical presentation, laboratory findings and outcome of a relatively unselected group of patients with renal disease. The patients were obviously selected in as much as they underwent renal biopsy but in other respects they were random. They are representative of the type of patients referred for specialised renal

investigation and management from a population of approximately one million people.

Figure 3



Immunofluorescence to IgA in the glomerulus of a patient with Mesangial IgG/IgA disease demonstrating the detail which can be seen with modern immunofluorescence techniques.

Case 255 IgA x 350

II MATERIAL

1. INTRODUCTION

The material for this study was obtained from 300 consecutive renal biopsies examined in the Pathology Department of the University of Edinburgh. The criteria for inclusion in this study were, a) an adequate biopsy for study by light, immunofluorescence and electron microscopy, and b) the availability of adequate clinical details, particularly with regard to the presenting features.

The biopsy was considered to be adequate if at least five glomeruli were present in the specimens used for both light and immunofluorescence microscopy. In the majority of cases only one glomerulus was sectioned by electron microscopy, but in some patients further glomeruli were examined.

The clinical details obtained were the presenting features, serological and biochemical analyses, urine analysis and clinical examination. From such data it is possible to ascribe a disease syndrome to each patient (vide infra, II.3). However, the presenting syndrome does not give an accurate prediction of the underlying disease. For instance a patient with nephrotic syndrome may have a proliferative or membranous glomerulonephritis but could have some other cause such as amyloidosis, diabetes mellitus or scleroderma. Biopsy will provide a histological diagnosis but in some instances a final diagnosis will be only reached after consideration of clinical features and histology such as in Henoch-Schonlein purpura where a variable histological appearance is recognised. Thus for each patient

in this study there is a clinical diagnosis, histological diagnosis and final diagnosis.

In the majority of patients adequate follow-up details were obtained over an interval varying between two and four years.

The 300 consecutive renal biopsies were performed in 267 patients. In 42 patients a biopsy had, in addition, been examined prior to the commencement of this study. Twenty-two of these patients had received some form of therapy directed against their renal disease (steroids 13, steroids and cyclophosphamide 2, steroids and Azathioprine 2, Indomethacin 4, steroids and Indomethacin 2). In the remaining 20 patients no specific therapy other than diuretics or antibiotics had been employed. In 28 patients a repeat biopsy was carried out during the course of this study. In 11 of these patients some specific therapy had been instituted after the first biopsy, the second being performed in order to determine the effect of therapy (Indomethacin 6, steroids 3, steroids and Indomethacin 1, post hysterectomy 1). In 3 patients a second biopsy was performed following renal transplantation. For the purpose of this study patients who had biopsies performed before and after transplantation are considered as two separate cases. Four transplant patients had two biopsies of the transplanted kidney.

2. INDICATIONS FOR RENAL BIOPSY

Renal biopsy was performed for several different reasons:-

a) to establish the pathological diagnosis in patients who had signs indicative of primary renal disease,

b) to confirm the diagnosis and establish the degree of renal involvement in patients who were suspected of

having a systemic disease, (e.g. polyarteritis nodosa, systemic lupus erythematosus, etc.),

c) to establish the degree of renal involvement in patients with conditions known to involve the kidney such as diabetes mellitus,

d) to follow the natural history of a particular disease,

e) to determine the effect of therapy on a particular disease,

f) in transplant patients to gain information regarding rejection episodes.

In certain instances it is difficult to place a patient in one of these groups. Patients are frequently complex and sometimes it is difficult to disentangle the symptoms and signs caused by a systemic disease from those produced by its renal involvement. For instance, polyarteritis may present as acute renal failure and in addition have pulmonary involvement. However, patients with acute renal failure may have fluid overload and thus pulmonary oedema. In the above grouping patients have been placed in category b. when there is good clinical or laboratory evidence of systemic disease prior to renal biopsy. The distribution of patients is shown in Table 1. In two cases small pieces of normal renal cortex were obtained surgically during operations for urinary tract obstruction. The final diagnosis of patients in groups a, b and c is shown in Tables 2, 3 and 4 respectively. The final diagnosis was determined according to the criteria in II.5. a - u..

TABLE 1

INDICATIONS FOR RENAL BIOPSY

	Group	Biopsies
a.	To establish a diagnosis	162
b.	Suspected systemic disease	19
c.	Diseases known to affect the kidney	25
d.	Study of natural history	37
e.	Study effects of therapy	33
f.	Transplant	22
g.	Normal	2

TABLE 2

FINAL DIAGNOSIS OF PATIENTS IN WHOM RENAL BIOPSY WAS PERFORMED TO ESTABLISH THE NATURE OF THE UNDERLYING DISEASE

Diffuse proliferative glomerulonephritis	55
Rapidly progressive glomerulonephritis	5
Membranous glomerulonephritis	7
Mesangiocapillary glomerulonephritis	7
Focal proliferative glomerulonephritis	8
Mesangial IgG/IgA disease	2
Minimal lesion glomerulonephritis	16
Focal glomerulosclerosis	5
Henoch-Schonlein Purpura	11
Acute tubular necrosis	9
Disseminated Intravascular Coagulation	7
Hypertension/Malignant hypertension	17
Amyloidosis	4
Polyarteritis	1
Scleroderma	1
Subacute bacterial endocarditis	1
Diabetes Mellitus (pre-diabetes)	2
Malignancy associated nephrotic syndrome	1
Chronic pyelonephritis	1
Goodpasture's Syndrome	1
Orthostatic proteinuria	1

TABLE 3

FINAL DIAGNOSIS IN THOSE PATIENTS IN WHOM THE CLINICAL FINDINGS WERE INDICATIVE OF A SYSTEMIC DISEASE

Suspected Diagnosis		Final Diagnosis	
Scleroderma	1	Scleroderma	1
Systemic Lupus Erythematosus	6	Systemic Lupus Erythematosus	4
		Active Proliferative G.N.	1
		Diffuse Proliferative G.N.	1
Polyarteritis	10	Polyarteritis	8
		Focal Proliferative G.N.	1
		Diffuse Proliferative G.N.	1
Amyloidosis	2	Amyloidosis	2

TABLE 4

FINAL DIAGNOSIS IN THOSE PATIENTS IN WHOM RENAL BIOPSY WAS PERFORMED TO ASSESS THE DEGREE OF RENAL INVOLVEMENT IN A CONDITION KNOWN TO AFFECT THE KIDNEY

Diabetes Mellitus	11
Systemic Lupus Erythematosus	5
Nephrogenic diabetes insipidus	1
Fanconi Syndrome	1
Toxaemia of pregnancy	1
Wegener's granuloma	1
Partial lipodystrophy	1
Subacute bacterial endocarditis	1
Inappropriate A.D.H. secretion	1
Familial Juvenile Nephronophthiasis	1
Polyarteritis	1

3. DEFINITION OF CLINICAL TERMINOLOGY

The clinical syndromes were defined as follows:-

a. Acute Nephritis

This was considered when there was an acute onset of proteinuria, haematuria, hypertension, reduced urine volume and oedema occurring some 14 to 21 days after an infective illness. In some instances, however, not all these features were present.

b. Nephrotic Syndrome

This was applied to cases where the proteinuria diminished plasma albumin concentration sufficiently to result in peripheral oedema.

c. Asymptomatic Proteinuria

This was assigned to cases where the proteinuria was discovered as an incidental finding at some other medical examination, frequently an examination for insurance purposes. In addition the proteinuria was confirmed on more than one occasion and was not of the orthostatic type.

d. Asymptomatic Haematuria

This term was used for patients who were found to have haematuria as an incidental finding at a medical examination and where the patient was previously unaware of its presence.

e. Recurrent Haematuria

This syndrome was applied to those patients who had repeated attacks of macroscopic haematuria frequently following minor upper respiratory tract or other infections. The classical feature was that the haematuria occurred in immediate association with the respiratory tract infection and was not delayed some 14 to 21 days,

as in acute nephritis.

f. Henoch Schonlein Disease

The clinical picture in this group was one of a purpuric haemorrhagic rash over the lower limbs associated with haematuria. Arthropathy, abdominal pain and positive faecal occult blood were signs present to a variable extent in most patients.

g. Acute Renal Failure

This term was used when there was a clear indication of an acute reduction in urine output, combined with a steadily increasing blood urea and serum creatinine in a patient with no past history referable to the renal system. This was frequently associated with a surgical procedure but sometimes occurred with no significant preceding history.

h. Chronic Renal Failure

This label was applied where significant reduction in renal function was clearly of long standing. The duration was assessed on the basis of anaemia, renal osteodystrophy, neuropathy, skin manifestations, or other changes which could be attributed to long standing impairment.

i. Hypertension

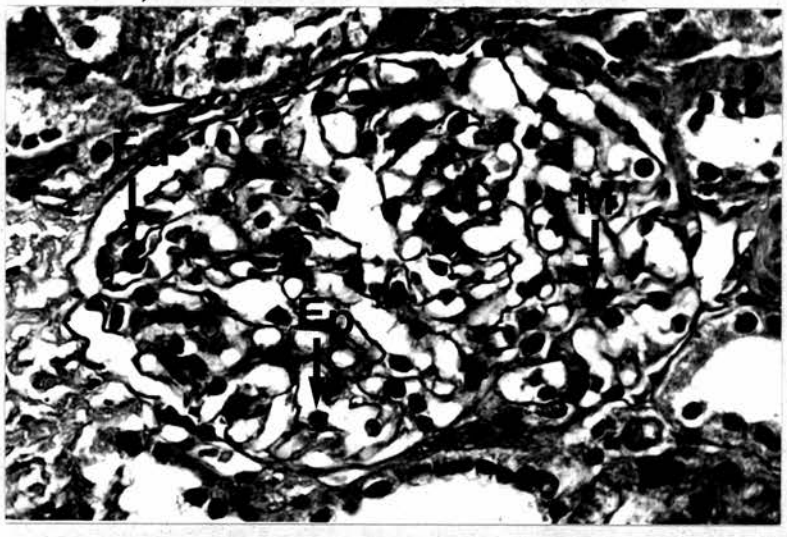
This was diagnosed when the pre-treatment diastolic blood pressure was consistently greater than 100 mm Hg. Malignant hypertension was identified when this finding was associated with papilloedema.

4. DEFINITION OF PATHOLOGICAL TERMINOLOGY

a. Normal

The glomerulus is composed of a tuft of capillaries which emerge at the hilum from the afferent arteriole and condense again at the hilum to form the efferent arteriole (Fig. 4). The capillaries are

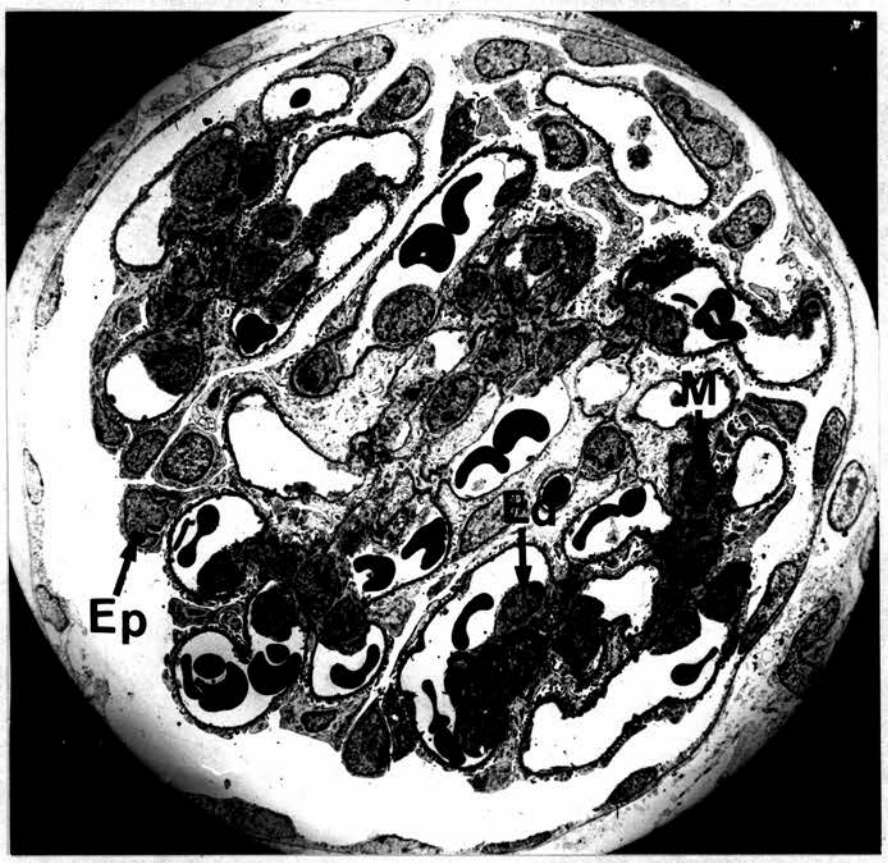
Figure 4



A normal glomerulus showing epithelial cells (Ep), mesangial cells (M) and endothelial cells (Ed).

H & E x 375

Figure 5



Electron micrograph of a normal human glomerulus showing epithelial cells (Ep), mesangial cells (M), and endothelial cells (Ed).

x 1050

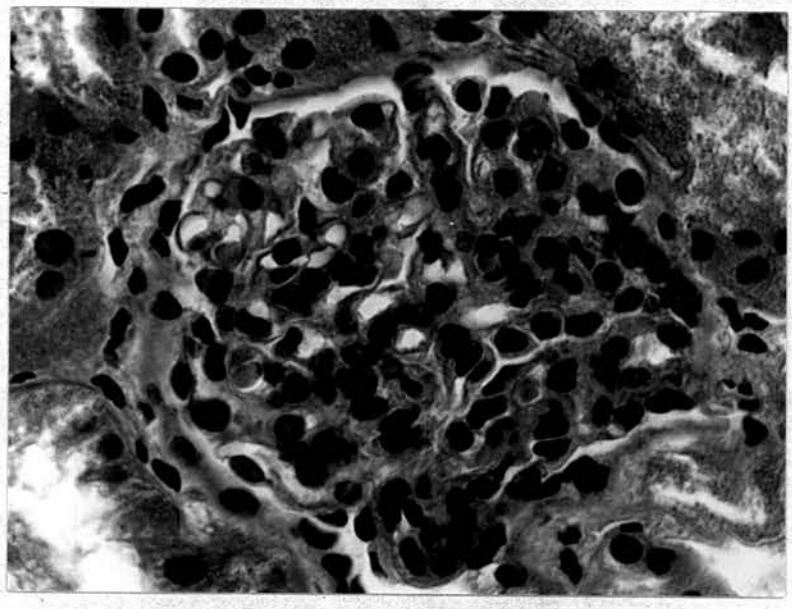
composed of endothelial cells, basement membrane and epithelial cells. The mesangial region at the junction of capillary loops comprise mesangial cells and matrix (Fig. 5).

b. Diffuse Proliferative Glomerulonephritis

In diffuse proliferative glomerulonephritis there is a diffuse proliferation of mesangial cells frequently associated with an increase in mesangial matrix. Occasionally endothelial cells may be swollen. In many specific conditions such as mesangiocapillary glomerulonephritis, systemic lupus erythematosus or Henoch Schonlein purpura there is often a diffuse proliferative glomerulonephritis, but in addition there is some other feature, either clinically or pathologically, which distinguishes the lesion and sets it apart (vide infra).

There is a spectrum of changes which in mild cases consists of only a minor increase in mesangial cells with little or no increase in matrix (Fig. 6). In such instances immunoglobulins, if present, are usually confined to the mesangium, and on electron microscopy there may be some electron dense material in mesangial matrix and occasionally in a subendothelial position. In moderate cases these changes are more marked; there may, in addition to mesangial cell increase, be evidence of endothelial swelling (Fig. 7). In these cases there is frequent deposition of products of inflammation detected by immunofluorescence and electron microscopy, in a subendothelial position in glomerular capillary walls and also in the mesangium. In proliferative glomerulonephritis of any severity, if it is due to circulating complexes, 'hump' deposits may be seen on the epithelial side of the basement membrane.

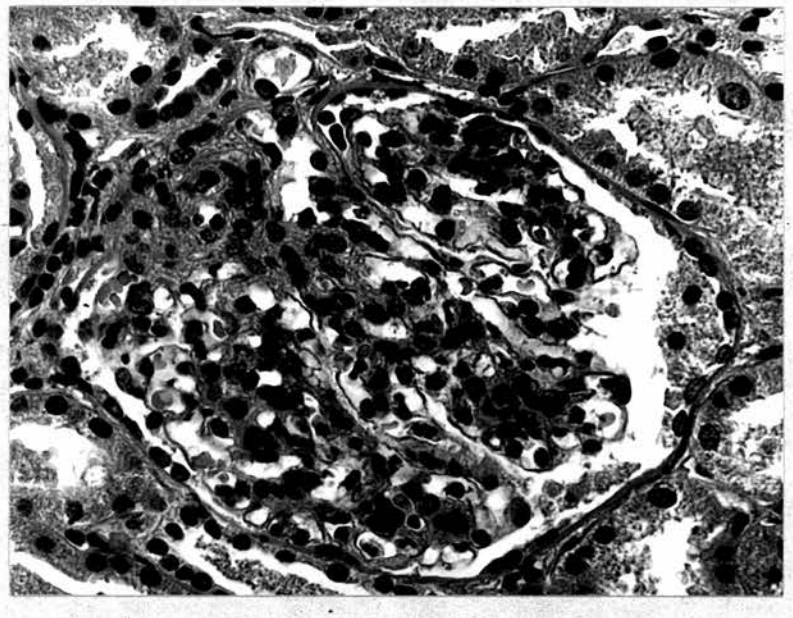
Figure 6



Mild proliferative glomerulonephritis showing the minor increase in mesangial cells with little increase in mesangial matrix.

Case 105 H and E x 525

Figure 7



Moderate proliferative glomerulonephritis with proliferation of mesangial cells. There are only a few polymorphonuclear leukocytes present.

Case 132 H and E x 350

Exudative diffuse proliferative glomerulonephritis denotes that type of proliferative glomerulonephritis in which there is obvious infiltration of polymorphonuclear leukocytes in the glomerular capillaries (Fig. 8), occasionally small crescents are also seen. This is considered to represent an 'acute' or 'active' phase of the disease as they are frequently associated with immunoglobulin and complement deposition. It is highly likely that the activation of the complement cascade resulting in the liberation of chemotatic factors results in the migration of polymorphs into the glomeruli.

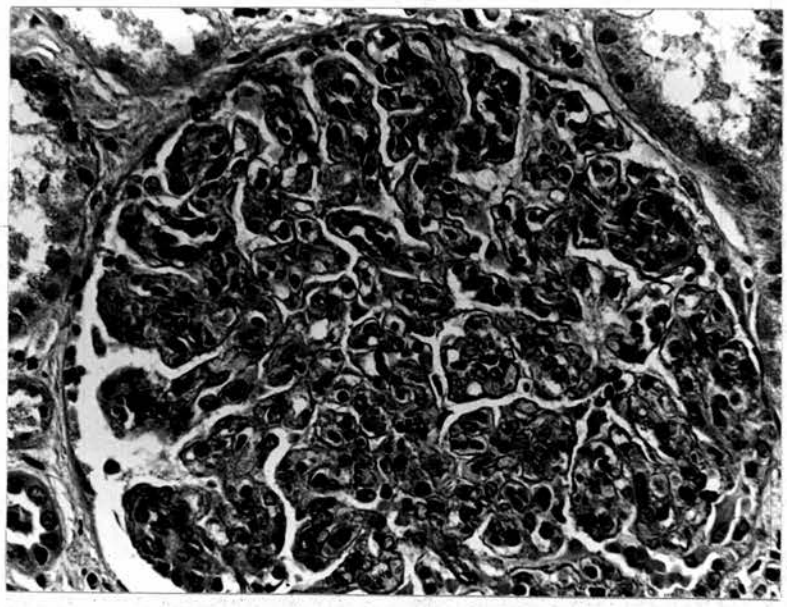
In progressive (sclerosing) proliferative glomerulonephritis there is an irregular increase in mesangial matrix associated with small crescents in some cases (Fig. 9). The hyalinised areas may be seen in some glomeruli only, but with time it is thought that they occur in an increasing number of glomeruli and by increasing in size progressively obliterate more and more glomeruli.

In summary, therefore, diffuse proliferative glomerulonephritis can be divided into mild, moderate or severe forms, exudative or progressive depending upon the various features mentioned above.

c) Rapidly Progressive (Crescentic) Glomerulonephritis

There may be little or no proliferation of glomerular tuft cells but there is parietal epithelial cell proliferation producing large, often circumferential crescents in 70% or more of the glomeruli (Fig. 10). On immunofluorescence microscopy there is little if any immunoglobulin deposition, and the most striking feature is the presence of large deposits of fibrin/fibrinogen in crescents. Electron microscopy shows grossly destorted glomeruli, sometimes with breaks in the basement membranes, and frequently it is difficult to

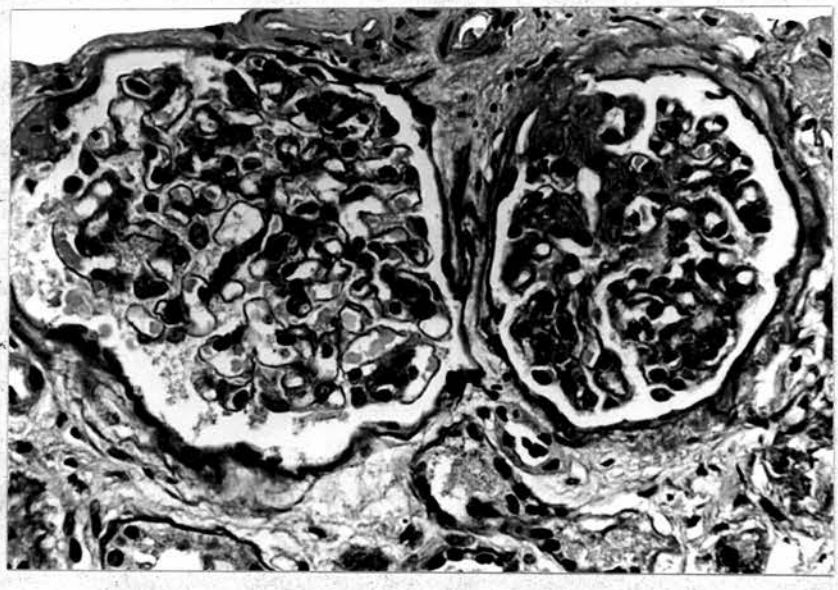
Figure 8



Exudative proliferative glomerulonephritis with marked proliferation of cells, endothelial cell swelling and obvious infiltration of polymorphonuclear leukocytes.

Case 109 H and E x 350

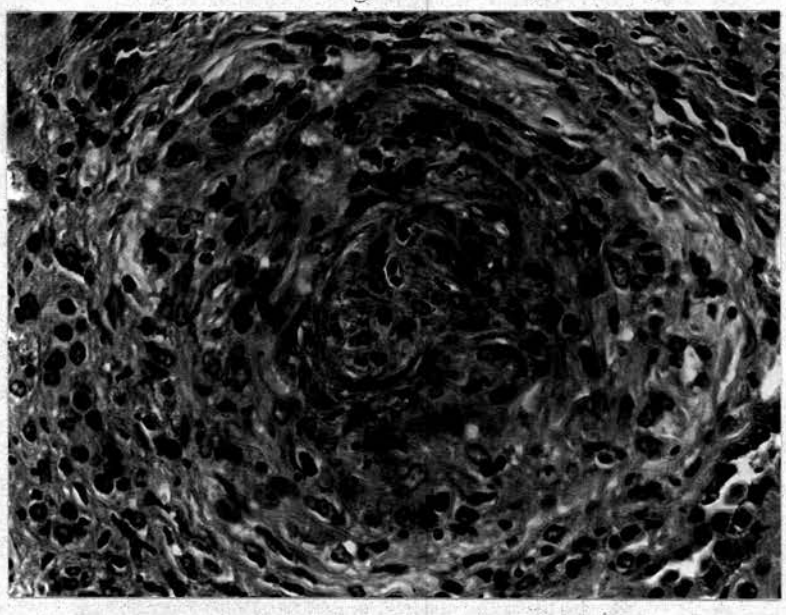
Figure 9



Progressive proliferative glomerulonephritis as demonstrated by a segmental increase in mesangial matrix. There is a minor increase in mesangial cells in both glomeruli

Case 220 H and E x 375

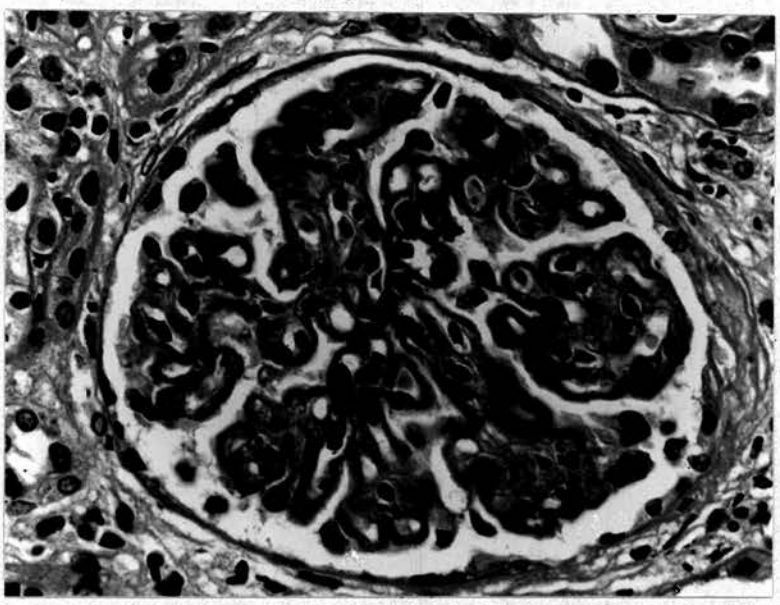
Figure 10



A large circumferential crescent surrounding the remnant of a glomerular tuft in a case of rapidly progressive (crescentic) proliferative glomerulonephritis.

Case 108 H and E x 350

Figure 11



The diffuse uniform increase in thickness of the glomerular capillary walls in a patient with membranous glomerulonephritis.

Case 165 H and E x 425

distinguish glomerular structure as opposed to crescent.

d. Membranous Glomerulonephritis

In membranous glomerulonephritis there is a diffuse uniform and hyaline thickening of the capillary basement membrane in all glomeruli (Fig. 11). In some cases there may be a minor proliferation of mesangial cells.

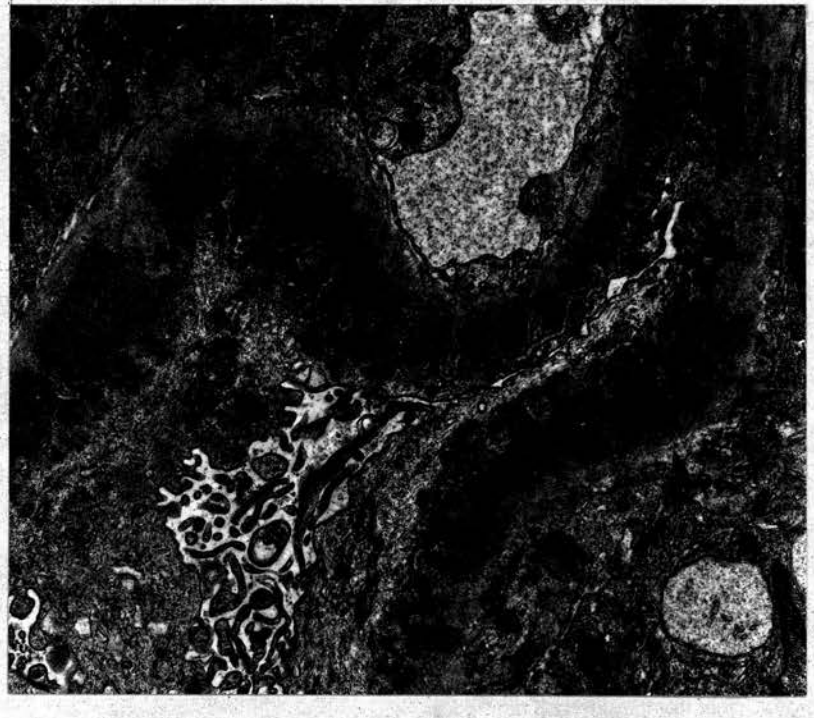
On electron microscopic examination small rounded or hump-shaped subepithelial deposits are seen on the capillary basement membranes (Fig. 12). In later stages epithelial cytoplasmic invagination and vacuolation of the basement membrane is often seen. By immuno-fluorescence microscopy the deposits are frequently shown to be immunoglobulins and complement (Fig. 13).

e. Mesangiocapillary Glomerulonephritis

The classical histological appearances are of a proliferation of mesangial cells with great increase of mesangial matrix so that capillary lumina are reduced to peripheral slits and glomeruli appear more solid than normal. Frequently the glomerular tuft is lobular. (Fig. 14). Mesangial cytoplasm can be seen in many capillaries between the endothelium and the basement membrane, and this may be demonstrable around the entire circumference of the capillary. There is often deposition of basement membrane-like material, possibly mesangial matrix, between this tongue of mesangium and the overlying endothelial cell to give the impression on light microscopy of a double basement membrane (tram-line effect) (Fig. 15). In some cases there is an intense staining of the glomerular basement membrane particularly with periodic acid Schiff stain (Fig. 16).

On electron microscopy two varieties of this disease can be

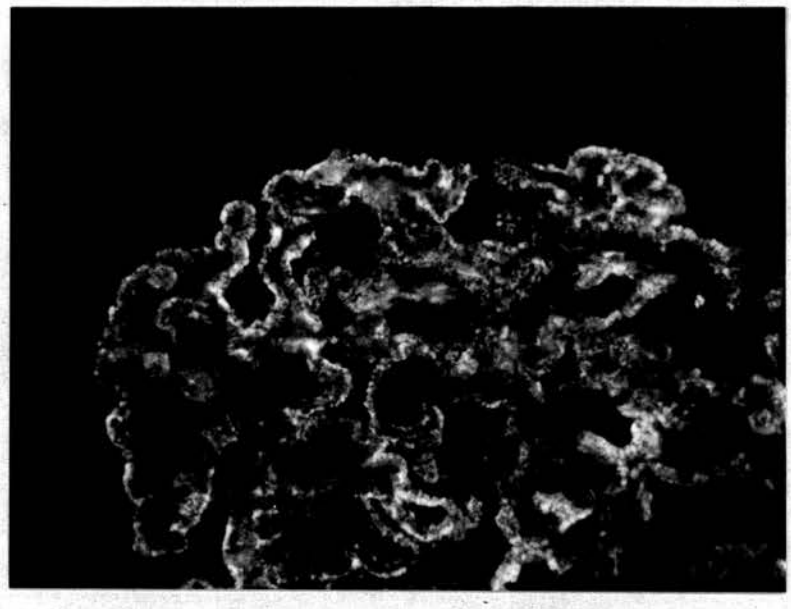
Figure 12



Electron micrograph in membranous glomerulonephritis. There are subepithelial electron dense deposits along both capillary walls. In this field there is complete loss of pedicel structure.

x 7000

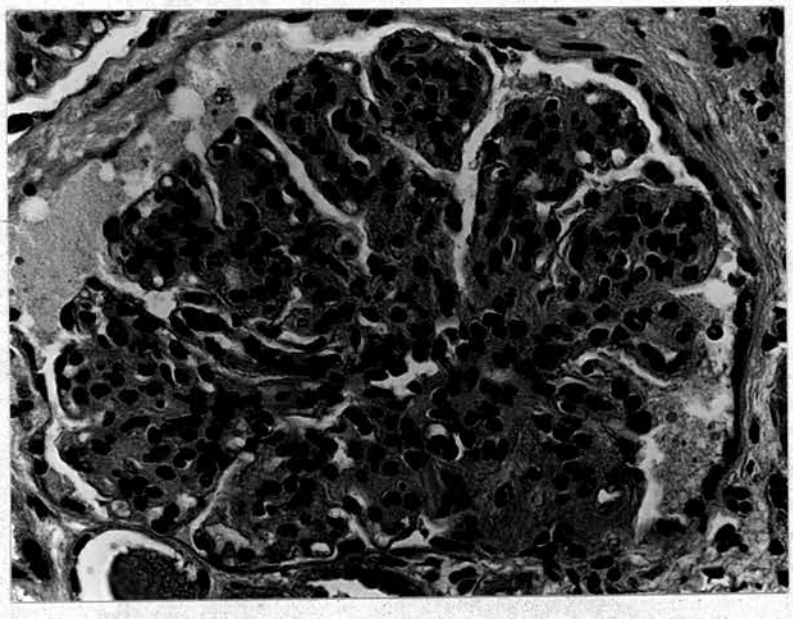
Figure 13



Immunofluorescence to IgG in a patient with membranous glomerulonephritis. The fluorescence has a typical granular appearance being most marked on the epithelial side of the membrane.

Case 264 IgG x 525

Figure 14



Mesangiocapillary glomerulonephritis demonstrating the proliferation of mesangial cells, the increase in mesangial matrix and the associated displacement of the capillary lumina giving a typical lobular appearance.

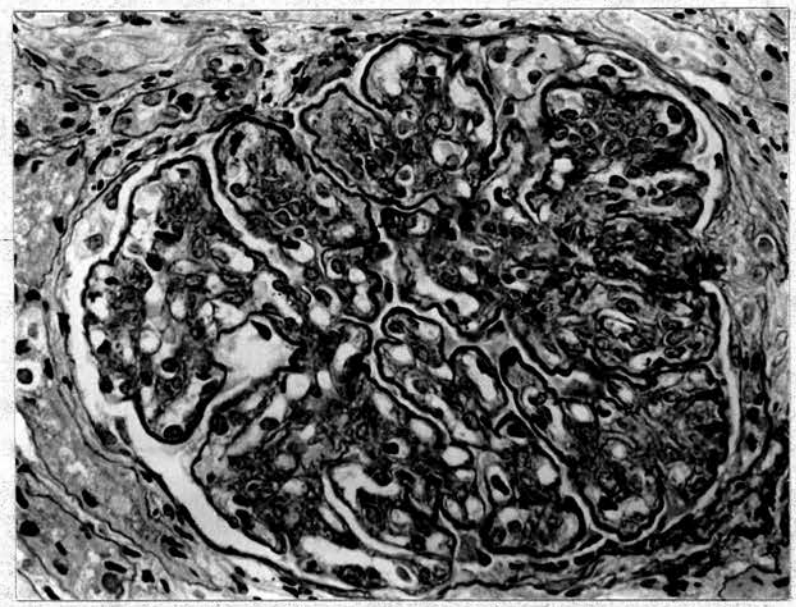
Case 226 H and E x 350

Figure 15

The double basement membrane (tram-line effect) of mesangiocapillary glomerulonephritis.

Case 226 H and E x 600

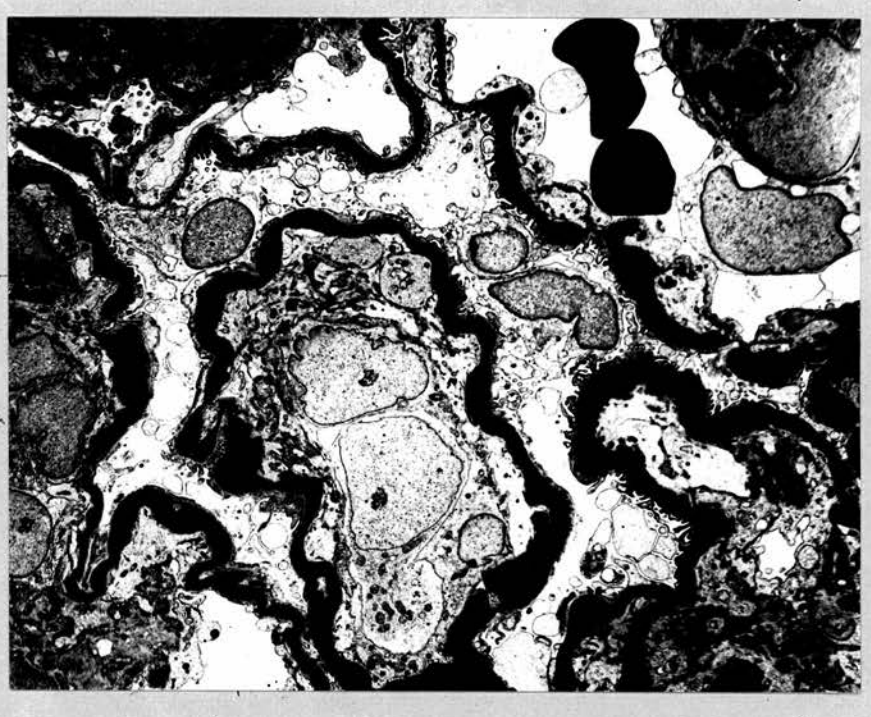
Figure 16



The intense staining of the dense deposit type of mesangiocapillary glomerulonephritis.

Case 149 P.A.S. x 375

Figure 17



Electron micrograph in the dense deposit type of mesangiocapillary glomerulonephritis demonstrating the intense electron dense, irregularly thickened basement membrane.

x 1750

defined, one, dense deposit dense, in which the basement membrane shows a dense intramembranous deposit and variably thickened (Fig. 17) and the other, subendothelial deposit type, where large, subendothelial deposits of a granular material of varying electron density are found (Fig. 18 and 19).

f. Focal Proliferative Glomerulonephritis

In this condition some but not all glomeruli are affected and commonly only some segments in these show proliferation of mesangial cells; endothelial cell swelling may be present (Fig. 20), and often some fibrin is seen.

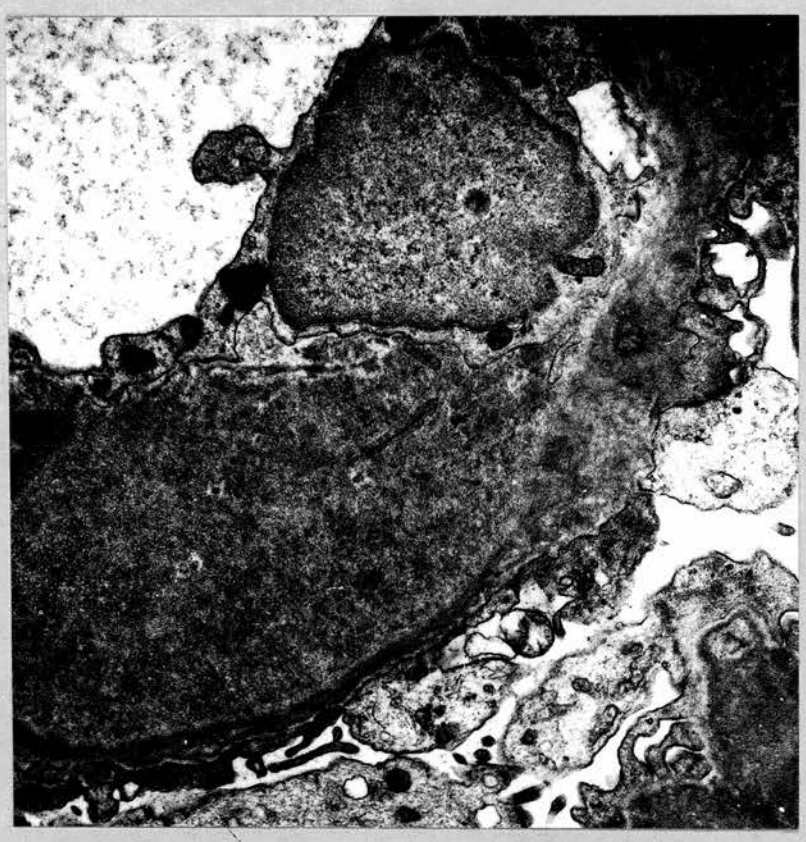
g. Mesangial IgG/IgA disease

This condition can be diagnosed conclusively only by immunofluorescence microscopy. On light microscopy the appearances may be of a mild proliferative glomerulonephritis which may be diffuse, focal or segmental, involving mesangial cells with moderate increase of mesangial matrix and prominence of mesangial regions (Fig. 21). In some cases no actual cellular proliferation is seen, but there is increase in mesangial matrix, diffuse, focal and/or segmental. By immunofluorescence microscopy a diffuse deposition of immunoglobulins, predominantly IgG and IgA, is seen in mesangial regions (Fig. 22). In addition there is sometimes some deposition of complement and fibrin/fibrinogen in a similar distribution. Little or no immunofluorescence is present elsewhere within the glomerulus.

h. Minimal Lesion Glomerulonephritis

In minimal lesion glomerulonephritis the light microscopy appearances are almost or completely normal. In some cases, minor to moderate degrees of mesangial cell proliferation are seen (Fig. 23).

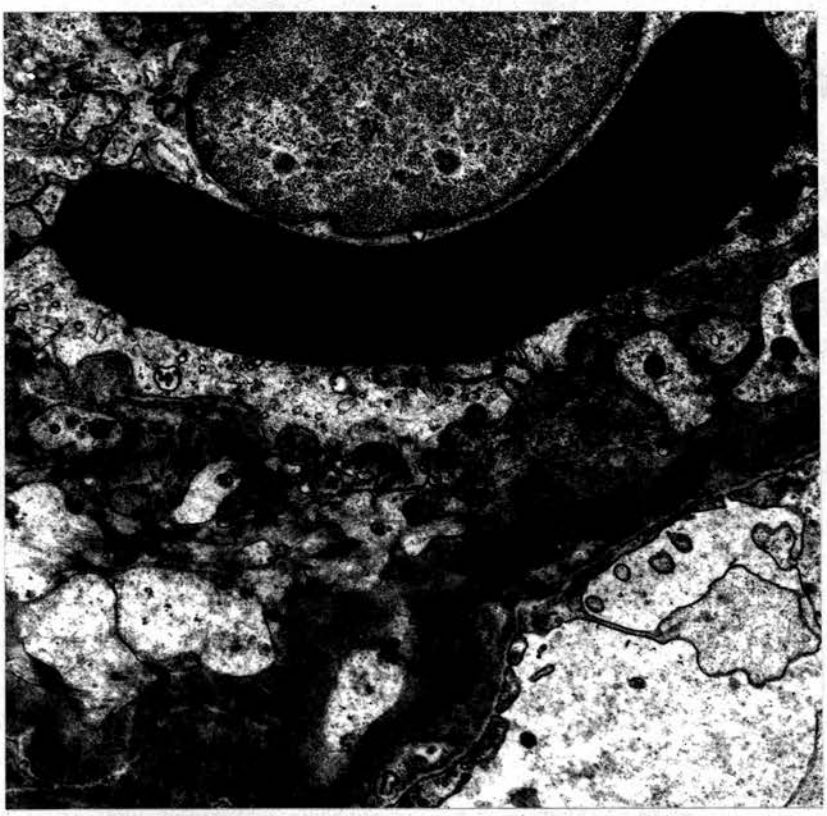
Figure 18



Electron micrograph of a segment of glomerular capillary wall showing a large granular subendothelial deposit. There is loss of pedicel structure.

x 10,500

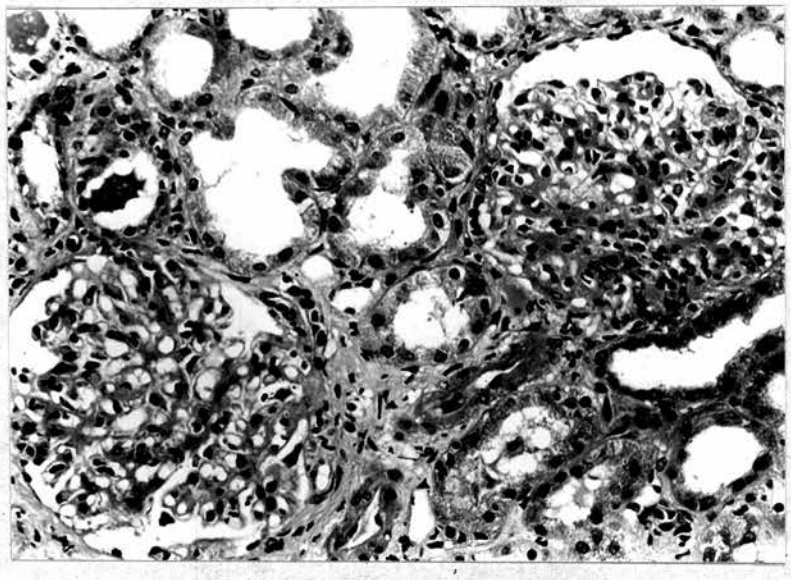
Figure 19



Electron micrograph of part of a capillary wall from a case of subendothelial deposit type of mesangiocapillary glomerulonephritis. Deep to the basement membrane mesangial cytoplasm can be seen and between this and the endothelium there is an irregular layer of mesangial matrix. The two layers (i.e. of basememt membrane and mesangial matrix) give the double contour effect.

x 7000

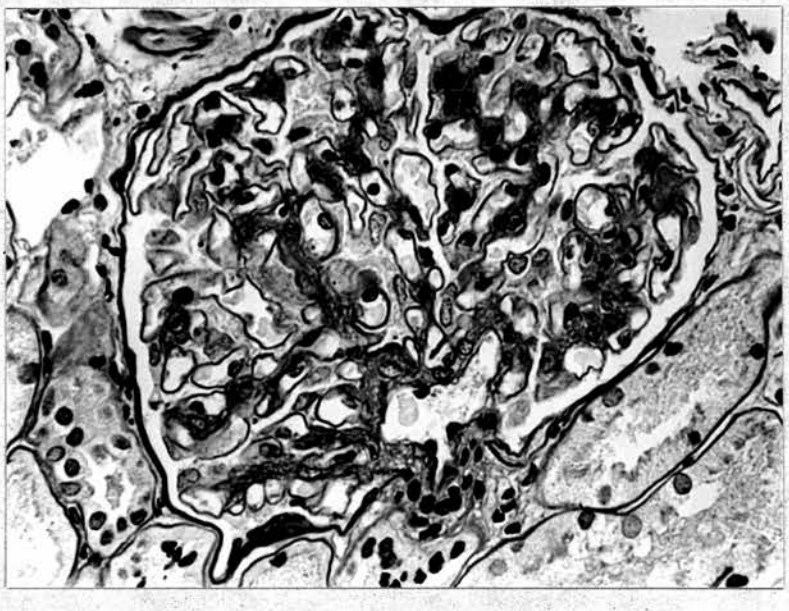
Figure 20



Focal proliferative glomerulonephritis in which the proliferation is most marked in one segment of one glomerulus, the remainder and the other glomerulus being relatively spared.

Case 282 H and E x 275

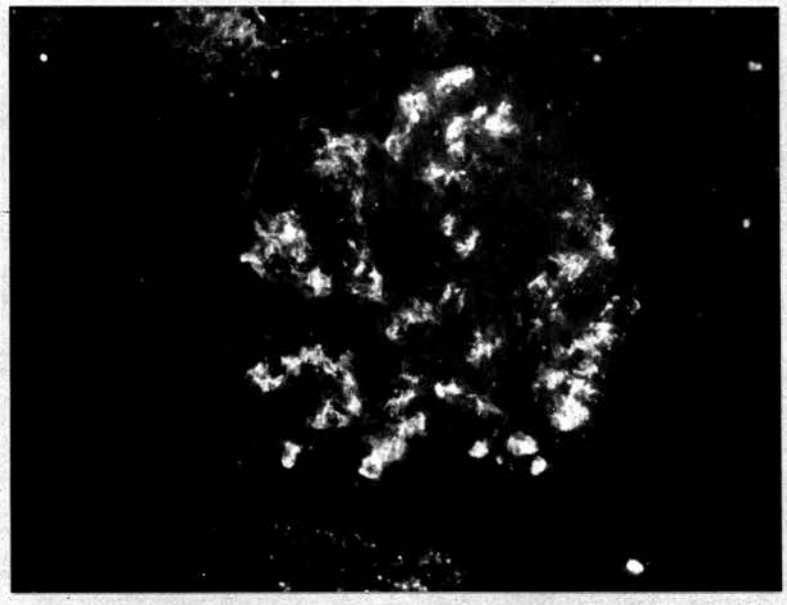
Figure 21



The minor proliferation of mesangial cells with increase in mesangial matrix in mesangial IgG/IgA disease.

Case 43 P.A.S. x 375

Figure 22

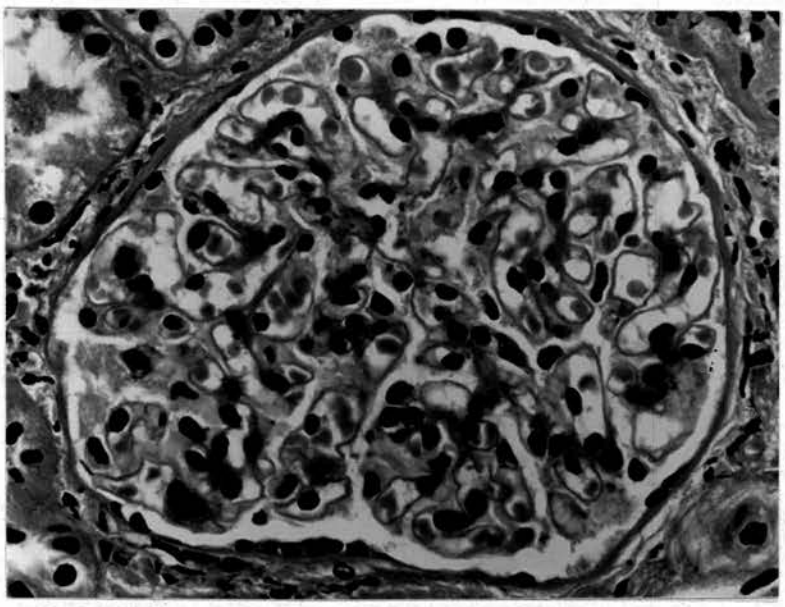


Immunofluorescence to IgA in mesangial IgG/IgA disease where the immunofluorescence is almost completely localised to the mesangium.

(See also Fig. 3).

Case 176 IgA x 350

Figure 23



Minimal lesion glomerulonephritis with only a minor proliferation of mesangial cells.

H and E x 425

On electron microscopy there is variable loss of the pedicel structure of the epithelial cells, the cytoplasm of which lies in a continuous layer over the basement membrane (Fig. 24). In addition, minor mesangial cytoplasmic activity is often demonstrable and there may even be a few small, dark, subendothelial deposits (Fig. 25). In the majority of cases, no specific immunofluorescence is seen in glomeruli, but occasionally a very little IgM and C3 may be observed (Fig. 26).

i. Focal Glomerulosclerosis

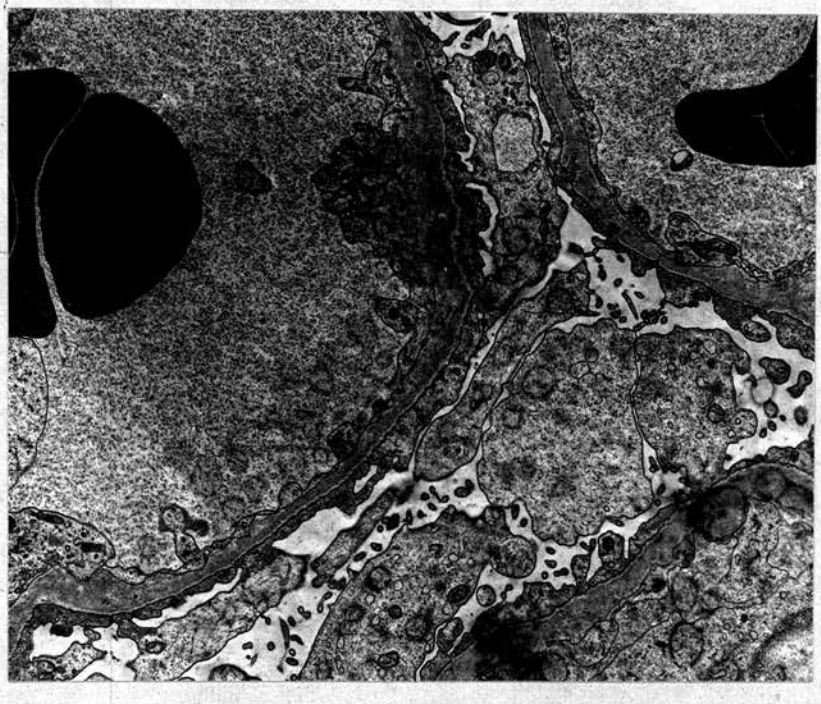
On light microscopy there is a focal and/or segmental hyalinisation of glomeruli (Fig. 27) which in the early stages of the disease is manifest only in juxtamedullary glomeruli. However, with time there is a progressive involvement of more superficial glomeruli.

j. Acute Tubular Necrosis

In acute tubular necrosis the predominant feature is seen in the tubules and the interstitium (Fig. 28). The basement membrane of the tubules shows focal disruption, and there may be apparent anastomosis between tubules and adjacent capillaries. Occasional tubular cells may show necrosis and desquamation. Many tubular lumina are dilated; if regeneration is occurring the epithelial cells are flattened and occasional mitotic figures are demonstrable in the epithelial cells. In the interstitial space there is an inflammatory cell infiltrate of plasma cells, lymphocytes and some polymorphs. There is frequently evidence of interstitial oedema and occasionally interstitial fibrosis.

The histological changes in the glomerulus are minor, although there may be the appearance of fibrin deposition in capillary loops.

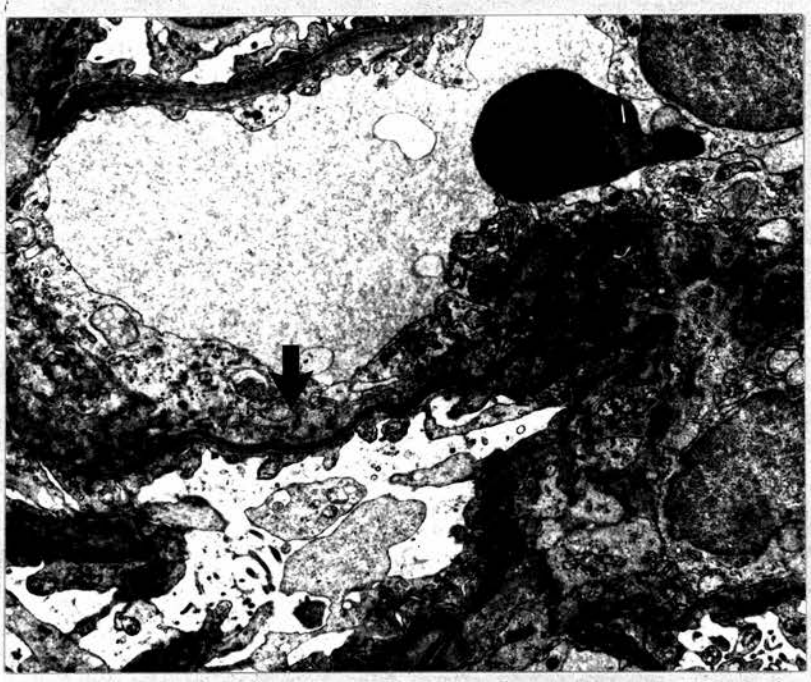
Figure 24



Electron micrograph in minimal lesion glomerulonephritis showing the loss of epithelial cell pedicel structure and the consequent "smearing" of the epithelial cell along the basement membrane.

x 7000

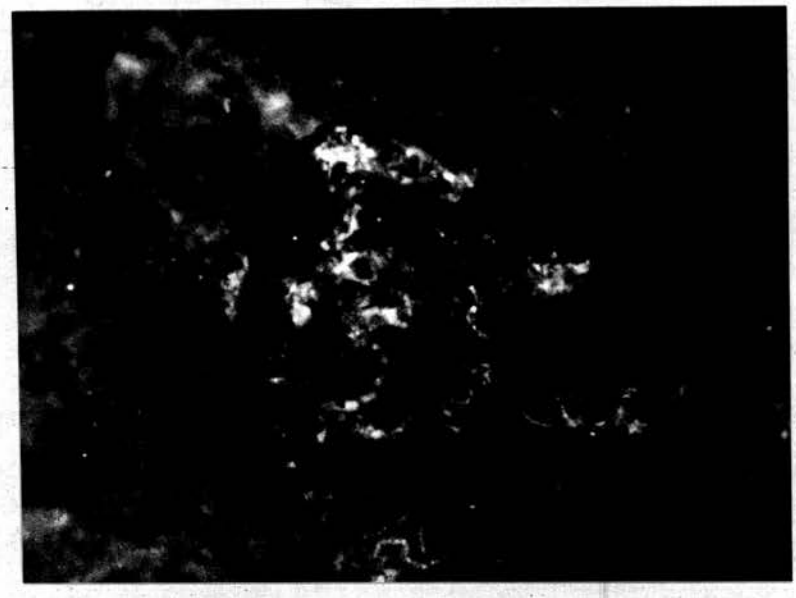
Figure 25



Electron micrograph in minimal lesion glomerulonephritis demonstrating minor mesangial activity and some subendothelial deposits (arrow).

x 2625

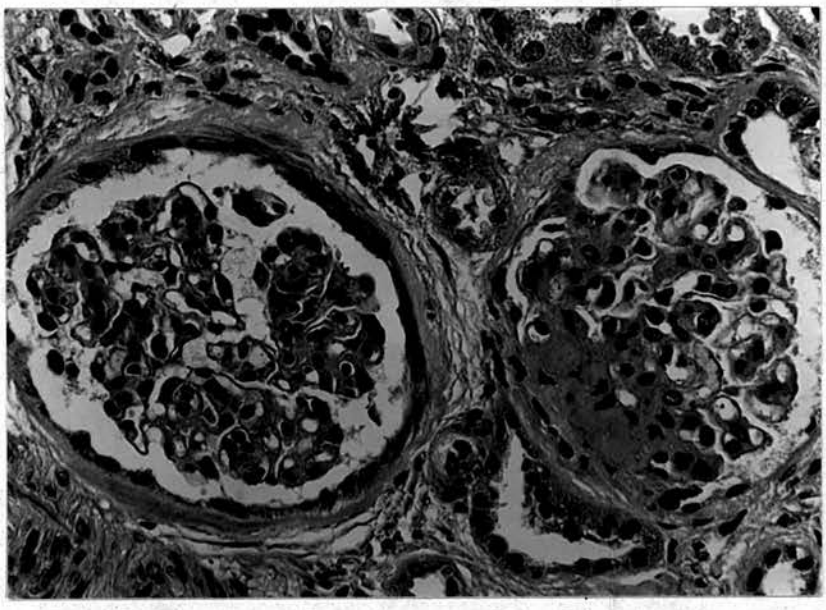
Figure 26



Immunofluorescence to C_3 in minimal lesion glomerulonephritis showing a very small amount of granular deposits in the glomerular capillary walls.

Case 146 C_3 x 350

Figure 27



Segmental hyalinisation in one glomerulus with sparing of an adjacent glomerulus in a patient with focal glomerulosclerosis.

Case 205 H and E x 350

Occasionally there is endothelial cell swelling and in some instances the mesangial regions appear enlarged.

k. Disseminated Intravascular Coagulation

In disseminated intravascular coagulation the predominant lesion is the appearance of MSB-positive material, judged to be fibrin, in glomerular capillaries and often afferent arterioles (Fig. 29). This frequently stains positive also with the picro-Mallory method. In addition there may be a considerable degree of acute tubular necrosis. Some glomeruli may be relatively spread, showing only some endothelial swelling.

l. Systemic Lupus Erythematosus

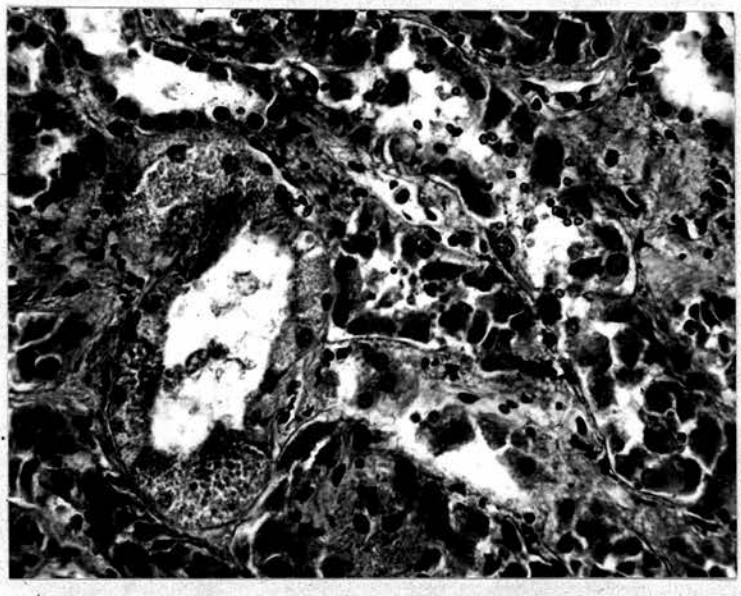
There is no single histological appearance that is pathognomonic for this condition. In the glomerulus there may be focal or diffuse mesangial cell proliferation, fibrin thrombi and focal 'fibrinoid' change ('wire-looping') in capillary walls (Fig. 30). Rarely haematoxyphil bodies may be seen.

On electron microscopy the characteristic lesion is the subendothelial deposition of dark granular material (Fig. 31) which by immunofluorescence stains for complement, immunoglobulins and fibrin/fibrinogen. Similar material may be seen in other situations in or on the basement membrane, and subepithelial 'hump' lesions are sometimes present.

m. Polyarteritis

The changes in polyarteritis nodosa can be extremely variable. In the 'microscopic' form there may be a variable focal proliferation of mesangial and possibly endothelial cells, but there may also be diffuse proliferation of these cells; there is often little or no

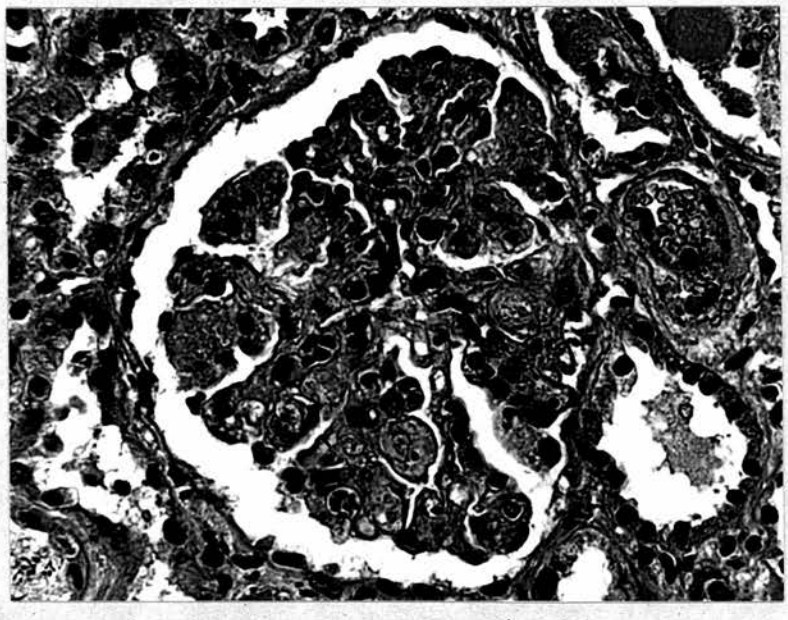
Figure 28



A few tubules show disruption of their basement membrane and associated degenerative changes in tubular cells. In one tubule, cut longitudinally, red blood cells can be seen in the tubular lumen indicating a tubulo-capillary anastamosis.

H and E x 250

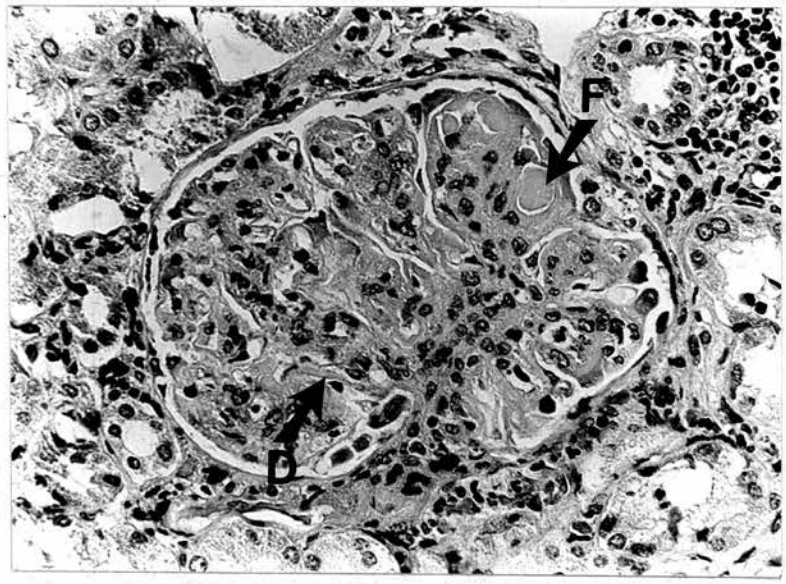
Figure 29



Thrombi in glomerular capillary loops and the lumen of an afferent arteriole in a patient with disseminated intravascular coagulation.

H and E x 350

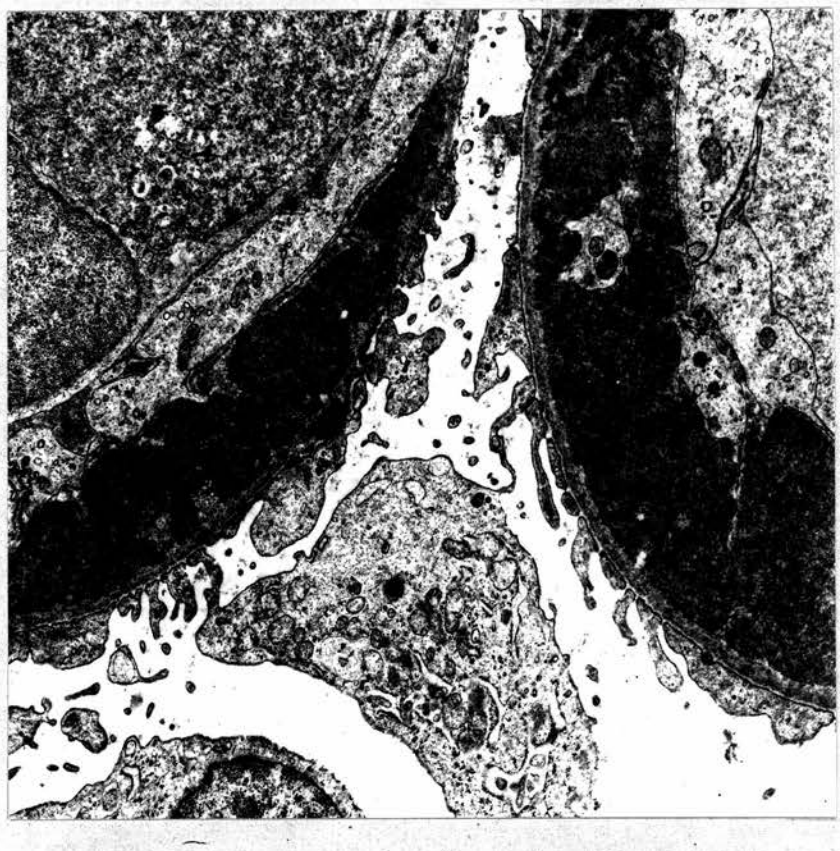
Figure 30



Segmental mesangial cell proliferation is seen and some capillary walls are thickened probably by subendothelial deposits (D). Two intraluminal fibrin plugs are visible. (F).

H and E x 300

Figure 31



Electron micrograph of dark subendothelial deposits in systemic lupus erythematosus.

x 7000

arteritis of large vessels (Fig. 32). However, in this 'nodosa' form there is frequently an infiltration of arterial walls with acute inflammatory cells, sometimes perivascular cuffing with chronic inflammatory cells and occasionally a granulomatous arteritis with giant cells in or around arterial walls. (Fig. 33). In addition, arteries and arterioles may show 'fibrinoid' necrosis of their walls and intimal proliferation may be so extensive as to occlude the lumen. In the glomerulus, cellular proliferation may be accompanied by crescent formation.

n. Scleroderma

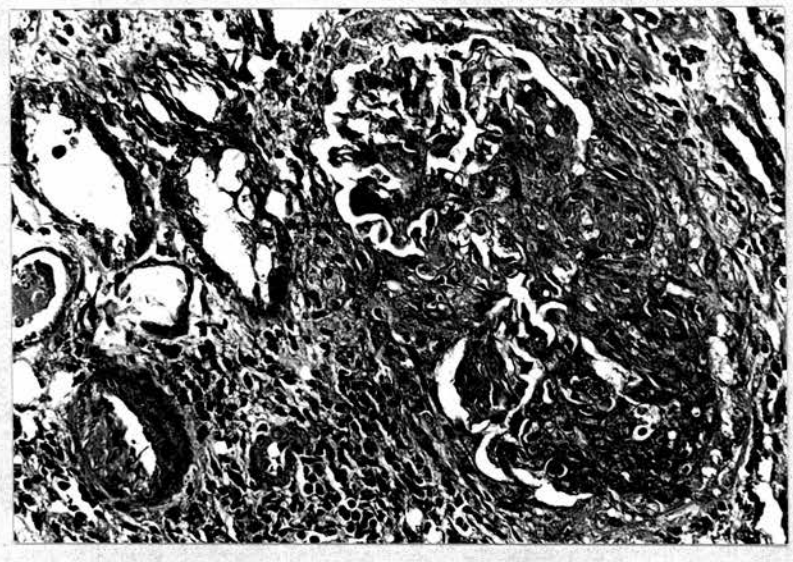
In scleroderma the predominant feature is an irregular mucinous subintimal accumulation in arterioles and arteries. This can reduce considerably the calibre of the lumen (Fig. 34). The relatively acellular intimal material stains metachromatically with toluidine blue, and similar stains. In addition, interlobular arteries and afferent arterioles may show 'fibrinoid' change in their walls and similar MSB-positive material may be seen focally in some glomeruli. Small infarcts of tubular tissue may be demonstrable.

o. Diabetic Glomerulosclerosis

In diabetic glomerulosclerosis there are two main histological groups, nodular and diffuse. In addition two other findings are characterised namely the capsular drop and the fibrin cap (see Fig 86).

In nodular diabetic glomerulosclerosis there are round, homogeneous, eosinophilic foci of varying size in the central part of lobules towards the periphery of the glomerular tuft, (Fig. 35). The nodules are formed by the deposition of accumulation of hyaline or basement-membrane-like material in the mesangium.

Figure 32



A glomerulus from a patient with microscopic polyarteritis.

A large segment of this glomerulus shows necrosis with intracapillary fibrin deposition. Adhesion between the affected part of the tuft and reactive cells of Bowman's capsule is seen. The surrounding intertubular tissue is oedematous and contains many inflammatory cells.

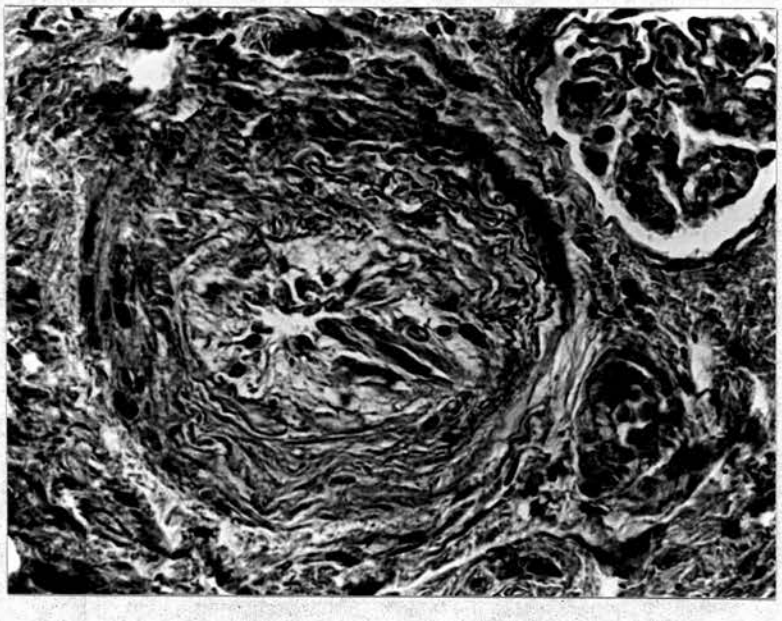
Case 254 H and E x 275

Figure 33

This field shows an arcuate artery the wall of which is completely destroyed by fibrinoid change. In the outer layers numerous acute inflammatory cells are seen. The glomeruli show no specific changes. The vascular changes are typical of polyarteritis nodosa.

H and E x 80

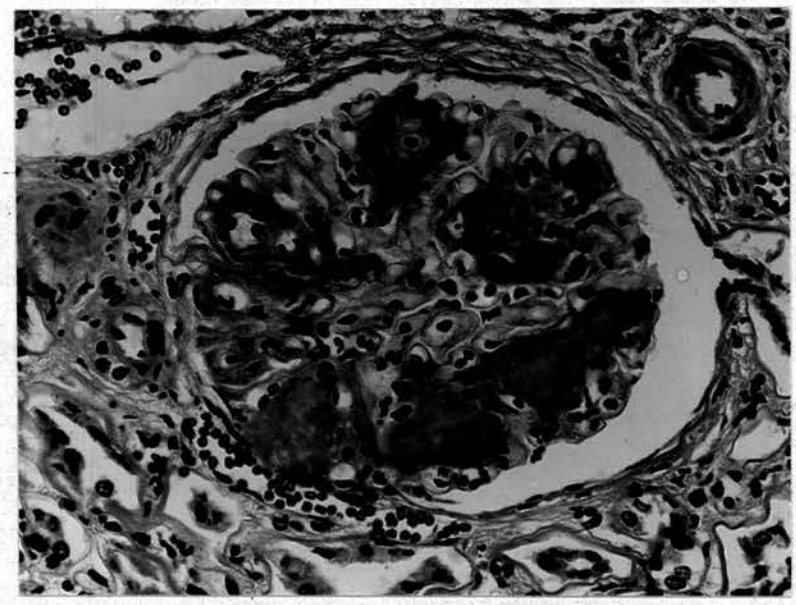
Figure 34



The subintimal accumulation of mucinous material in a small artery of a patient with scleroderma.

Case 235 P.A.S. x 425

Figure 35



Nodular diabetic glomerulosclerosis with round, homogeneous foci in the central part of lobules.

H and E x 275

In the diffuse form there is a diffuse or widespread accumulation of eosinophilic material in the mesangium throughout the glomeruli. This is associated with a uniform thickening of the capillary walls to a variable extent (Fig. 36).

The capsular drop is a nodule of eosinophilic material on Bowman's capsule between the basement membrane and the parietal epithelial cells. The fibrin cap is similar in staining properties but is situated in the concavity of a capillary loop usually at the periphery of the tuft.

The nodular form of diabetic glomerulosclerosis appears to be confined to patients with diabetes mellitus; the diffuse form is less pathognomonic and is similar to findings in certain types of glomerulonephritis and arteriosclerosis. Neither the capsular drop nor the fibrin cap is specific.

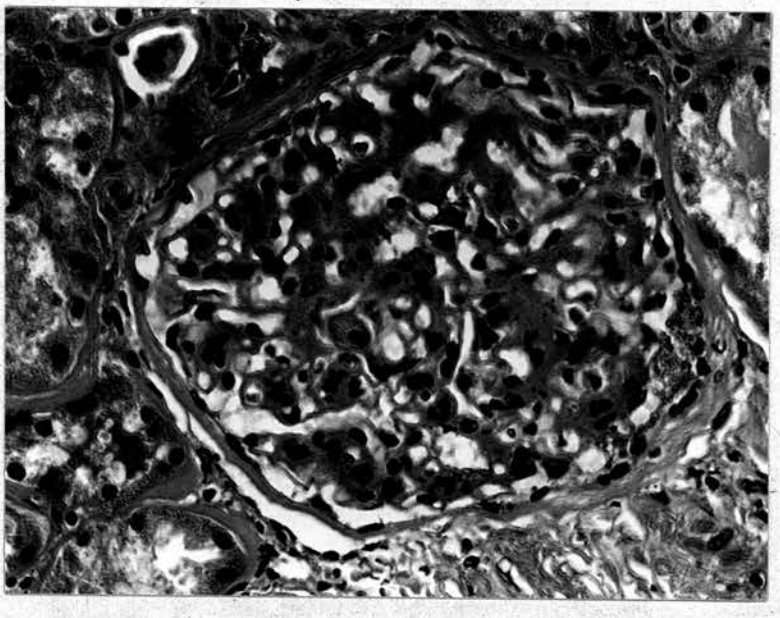
p. Amyloidosis

In renal amyloidosis the predominant changes are frequently in the glomeruli. The glomerulus may be completely hyalinised, but in the earlier stages shows the deposition of a pale homogeneous material which is initially present subendothelially and within the mesangium. This may increase to produce a nodular pattern (Fig. 37). A similar material is frequently seen in arteriolar walls and in the basement membrane of tubules.

This pale homogenous material stains positively in a Congo Red stain; this is most specific under ultra-violet light when typical fluorescence is seen (Fig. 38).

By electron microscopy the deposited material is seen to consist of large and irregularly orientated fibrils, present in subendothelial

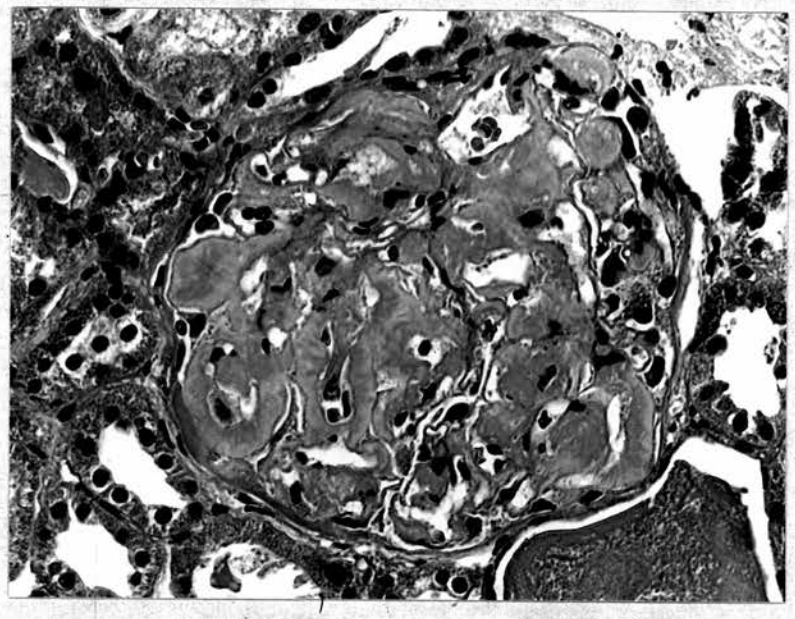
Figure 36



Diffuse diabetic glomerulosclerosis showing widespread accumulation of material in the mesangium and a fairly uniform increase in the thickness of capillary walls without proliferation of mesangial cells.

Case 107 H and E x 350

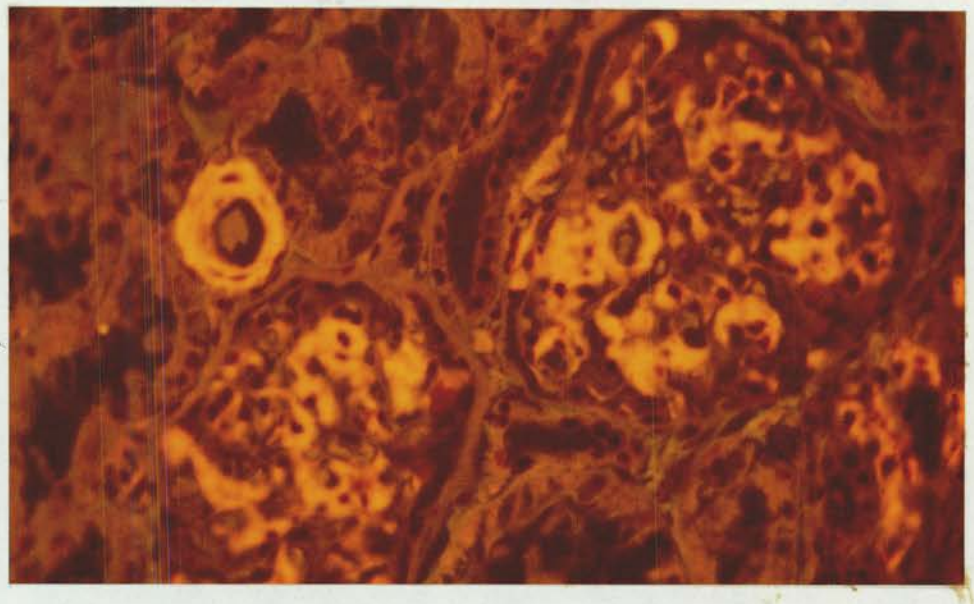
Figure 37



The deposition of homogeneous material in the subendothelial region of capillary walls and the mesangium of a patient with amyloidosis.

P.A.S. x 350

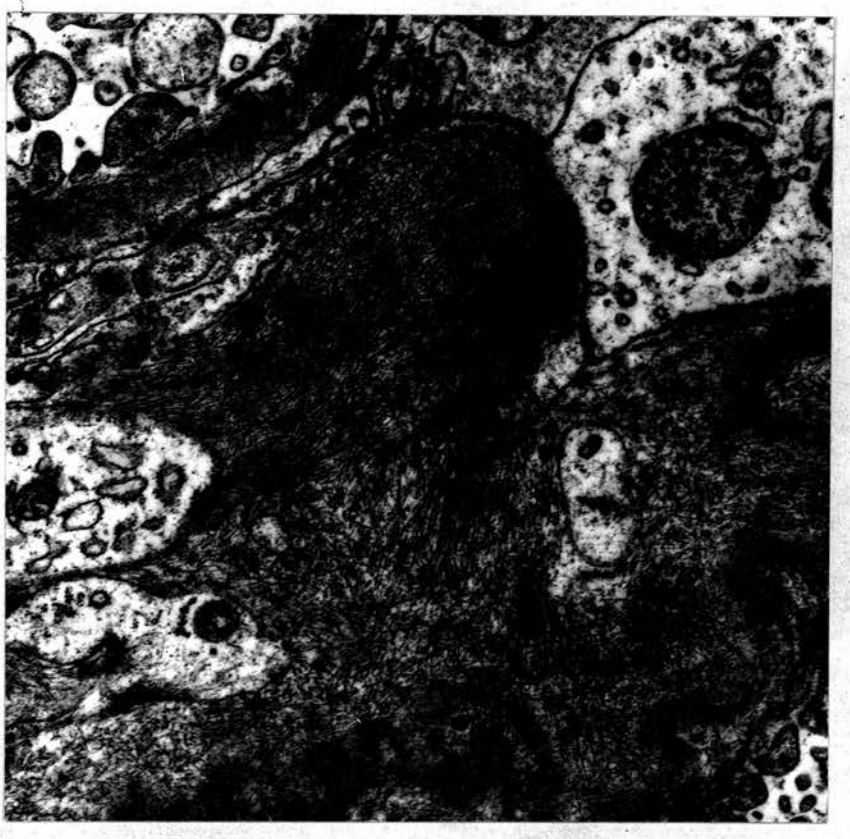
Figure 38



Fluorescence of Congo Red stain in amyloidosis demonstrating Congo Red positive material, presumably amyloid, in glomerular capillary walls, the mesangium and also in the walls of an arteriole.

Congo Red x 300

Figure 39



Electron micrograph in amyloidosis showing large, irregularly orientated, fibrils in the mesangium.

x 17500

and mesangial situations (Fig. 39).

5. CRITERIA FOR FINAL DIAGNOSIS

a. Introduction

The nomenclature of glomerular disease is confusingin the extreme as it employs a variable mixture of terms relating to clinical presentation, histological appearance and natural history. In this study the final diagnosis is that diagnosis which has been reached after all the clinical and laboratory details have been obtained but excludes information obtained at follow-up study. For example a patient with an acute nephritic illness, have a biopsy appearance of a mild diffuse proliferative glomerulonephritis and show clinical resolution over a period of time. In this study such a patient would be given a diagnosis of proliferative glomerulonephritis although, depending upon ones preference, the diagnosis could be just as easily termed acute glomerulonephritis, resolving proliferative glomerulonephritis or mild diffuse proliferative glomerulonephritis. The important part of any study is to define terminology clearly and then use such terminology consistently.

b. Proliferative Glomerulonephritis

Proliferative glomerulonephritis has been diagnosed on histological grounds. It denotes a disease syndrome characterised by proteinuria and/or haematuria and/or hypertension which histologically shows diffuse proliferation of mesangial cells to a variable extent (II.4.b), with no other features characteristic of a more specific disease.

At follow-up examination the term resolved proliferative glomerulonephritis was used where the biopsy had shown a diffuse proliferative glomerulonephritis, and where there was clinical evidence of a total

return to normal. Progressive proliferative glomerulonephritis has been used where there is again biopsy evidence of diffuse proliferative glomerulonephritis but in addition clear evidence at follow-up study of progressively deterioration in renal function.

c. Rapidly Progressive (Crescentic) Glomerulonephritis

Rapidly progressive glomerulonephritis is diagnosed in those patients in whom there is rapidly declining renal function and large circumferential crescents in 70% or more of the glomeruli on histological examination. (II.4.c).

d. Membranous Glomerulonephritis

This has been diagnosed on morphological grounds, as indicated in the previous section (II.4.d).

e. Mesangiocapillary Glomerulonephritis

This has been diagnosed on the morphological grounds indicated in the previous section (II.4.e).

f. Focal Proliferative Glomerulonephritis

This has been diagnosed on the morphological grounds indicated in the previous section (II 4.f).

g. Mesangial IgG/IgA Disease

This disease can be conclusively diagnosed only by immunofluorescence microscopy, as indicated in the previous section (II 4.g).

h. Minimal Lesion Glomerulonephritis

Minimal lesion glomerulonephritis has been diagnosed in patients who have proteinuria unassociated with either hypertension or haematuria and in whom steroid therapy produces a remission in the proteinuria. The proteinuria is highly selective, indicating the passage of small molecular weight proteins. It is a condition associated with frequent

relapses and remissions and often the relapses are associated with some minor febrile episode. The biopsy appearances by light, immunofluorescence and electron microscopy are detailed in II 4.h.

i. Focal Glomerulosclerosis

At initial presentation this syndrome is clinically indistinguishable from minimal lesion glomerulonephritis except that steroids may not be effective in producing a remission of the proteinuria. As the disease progresses there may be deterioration in renal function and the development of chronic renal failure.

The diagnosis of this condition may present difficulties as in the initial stages only juxtamedullary glomeruli are involved, so that if these are not included in the biopsy specimen the diagnosis may be missed (II 4.i)..

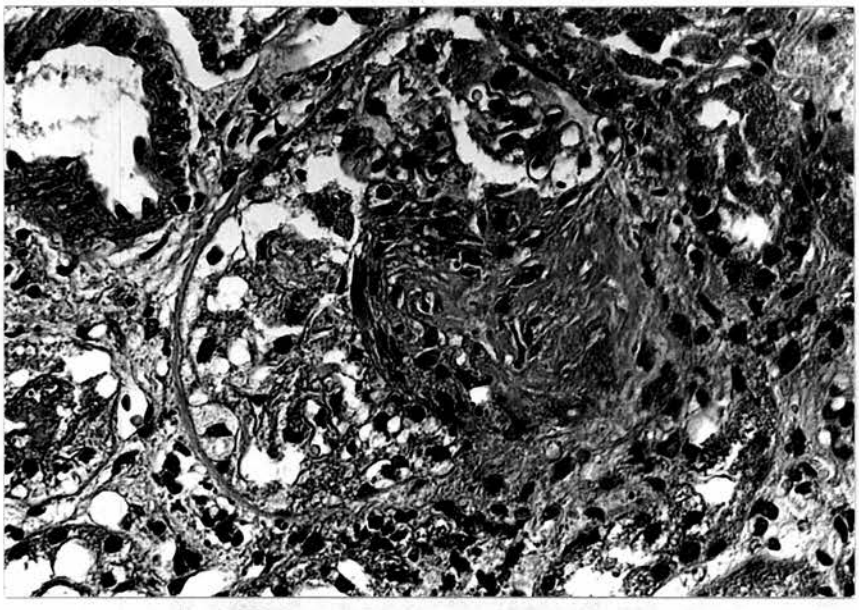
j. Henoch Schonlein Disease

This diagnosis is mainly on clinical grounds. The appearances are of a purpuric haemorrhagic rash over the lower limbs associated with haematuria and/or arthropathy and/or abdominal pain and/or hypertension. Biopsy findings are usually of a focal proliferative glomerulonephritis of variable degree (Fig. 40), often associated with some fibrin deposition, and immunofluorescence microscopy evidence of IgA deposition. Small crescents are frequently associated with the proliferative foci and in some instances the crescents may be circumferential.

k. Acute Tubular Necrosis

These patients present with acute renal failure and on investigation no underlying primary glomerular disease is detected. Most frequently the syndrome is caused by a hypotensive episode associated with either

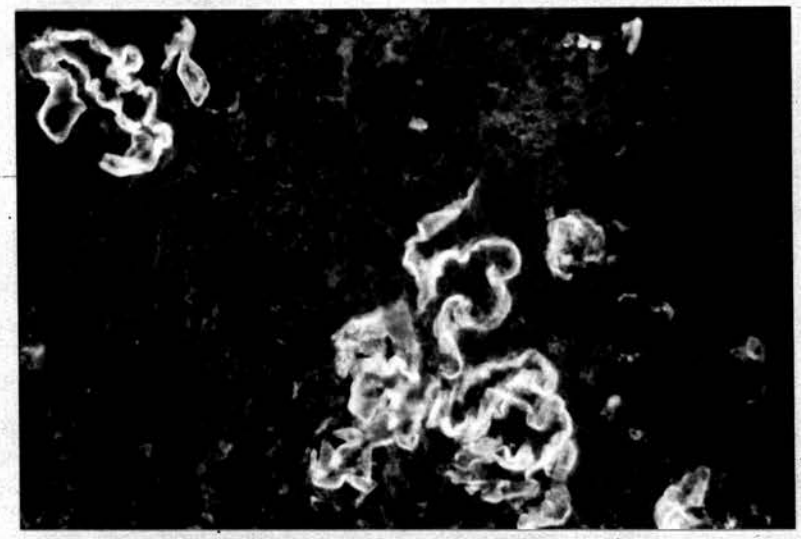
Figure 40



This is a glomerulus from a case of Henoch Schonlein disease. A large segmental "fibrinoid" lesion associated with some cellular proliferation is visible.

Case 40 H and E x 300

Figure 41



Linear deposition of IgG along the basement membrane of glomerular capillary walls in Goodpasture's Syndrome.

IgG x 300

blood loss or septicaemia. Renal function returns after a variable period of time but usually within 14 - 21 days of the original episode. The histological changes are described in II 4.j.

l. Disseminated Intravascular Coagulation

Clinically this condition can be suspected when there is evidence of excessive bruising, multiple small petachiac or difficulty in stopping bleeding from surgical wounds and venepuncture sites. Occasionally there is spontaneous bleeding from the gums or into the gastrointestinal, respiratory or genito-urinary tract. It is confirmed by the laboratory finding of a reduction in platelets and coagulation factors, associated with distorted red cells (helmet cells and schistocytes), free plasma haemoglobin and fibrin/fibrinogen degradation products in plasma. The syndrome is precipitated by a factor or factors which result in the intravascular deposition of strands of fibrin leading to widespread capillary occulusion and a haemorrhagic diathesis.

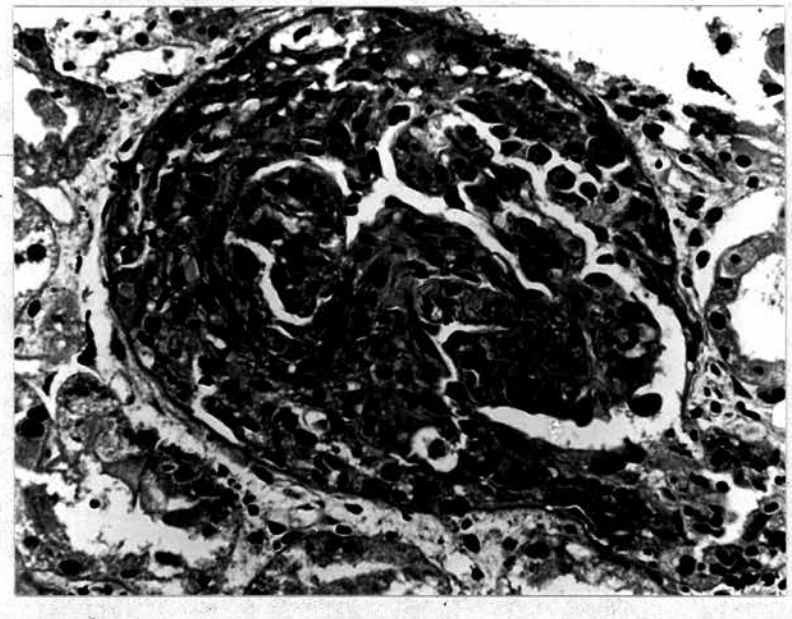
m. Goodpasture's Syndrome

This syndrome is characterised by haematuria and haemoptysis associated with a circulating anti-glomerular basement membrane antibody. Unfortunately in this series it was not possible to determine the presence of such an antibody in the serum, as techniques for its detection were not available. By immunofluorescence microscopy there is a linear staining of the glomerular basement membrane (Fig. 41) and light microscopy frequently shows the features of rapidly progressive or crescentic glomerulonephritis (Fig. 42).

n. Systemic Lupus Erythematosus

This diagnosis is made mainly on the serological evidence of a

Figure 42



Rapidly progressive (crescentic) glomerulonephritis in a patient with Goodpasture's Syndrome.

H and E x 300

circulating anti-nuclear factor (ANF) in the serum associated with a variable clinical picture of facial erythema, arthritis, pleuritic chest pain, pericarditis and Raynaud's phenomenon. This may be associated with a mild haemolytic anaemia, leukopenia and thrombocytopenia. As indicated previously there is no single pathognomic histological appearance (II 4.1).

o. Polyarteritis

This condition is characterised by some or all of the following symptoms; pleuritis chest pain, haematuria, weight loss, joint pain and anorexia. Symptoms are multisystem in origin and indicate a widespread arteritis process. The 'nodosa' type can be diagnosed with certainty histologically only by finding fibrinoid necrosis and infiltration of arterial and arteriolar walls with inflammatory cells. The 'macroscopic' type may show either a diffuse or more usually a focal proliferation of mesangial and endothelial cells with some fibrin deposition (II 4.m).

p. Scleroderma

This condition is frequently suspected in patients with Raynaud's phenomenon and dysphagia, but can be diagnosed histologically only by the finding of a) a thickened arterial intima containing mucinous material which stains metachromatically with certain dyes, and b) sometimes necrosis of arteriolar and interlobular arterial walls, together with variable resultant ischaemic changes (II 4.n).

q. Hypertension

This is diagnosed on clinical grounds as described previously (II 3.i). Malignant hypertension can be characterised additionally by the finding of arteriolonecrosis.

r. Diabetes Mellitus

This is diagnosed on clinical findings and the presence of an abnormal glucose tolerance test.

s. Chronic Pyelonephritis

This is diagnosed on the radiological findings of dilated and clubbed calyces associated with irregular contraction of the cortical area on intravenous pyelography. This may or may not be associated with any urinary tract symptoms, or abnormal urinary sediment.

t. Malignancy Associated Nephrotic Syndrome

This is confined to the rare finding of the nephrotic syndrome associated with the presence of a malignant tumour and the regression of the nephrotic syndrome and proteinuria with the removal of the tumour.

u. Orthostatic Proteinuria

This term is used to describe the finding of proteinuria present only after the patient has been in an upright posture for some time (usually 1 - 2 hours), urine tested after a period of recumbancy being free from protein.

III METHODS

1. Light Microscopy

The material was fixed in formol saline, alone or containing 1% mercuric chloride, and embedded in paraffin; 2 to 3 micron sections were routinely stained with haematoxylin and eosin, periodic acid Schiff, Martius Scarlet Blue and picro-Mallory methods. Silver methenamine, Congo Red and Toluidine Blue stains were employed if necessary.

2. Electron Microscopy

Small pieces of tissue approximately 2 mm cubed were placed in 1% osmium tetroxide or in 3% glutaraldehyde with subsequent fixation in osmium tetroxide. The material was embedded in araldite and sectioned at 40 nm; sections were stained with lead citrate and uranyl acetate and examined in an A.E.I. E.M. 6 or Corinth 275 electron microscope.

3. Immunofluorescence Microscopy

Fresh tissue was placed in a cold moist chamber and transported as rapidly as possible to the laboratory. The tissue was then suspended in approximately 1 ml of O.C.T. (Labtek) and rapidly frozen with solid carbon dioxide. Sections were cut at 2 to 3 microns in a Bright cryostat (Fig. 43) and fixed in 95% alcohol for 10 minutes. Following fixation the specimens were either exposed to FITC-conjugated antisera or stored at -20°C.

The immunofluorescence microscopy was carried out under the

Figure 43

Bright Cryostat

following schedule.

After the sections had been fixed for 10 minutes in 95% alcohol they were washed in phosphate buffered saline for 5 minutes. The slides were dried, and 2 drops of appropriately prepared antisera (vide infra) were placed over them. The sections were allowed to incubate at room temperature in a dark moist chamber for 30 minutes. After this time excess antisera was washed off with phosphate buffered saline. The slides were dried and mounted with a cover slip in dilute glycerine (1 : 9 glycerine : phosphate buffered saline). The sections were viewed on the same day with a Leitz Ortholux microscope (Fig. 44) using an H.B. 200 light source and B.G. 12 and B.G. 38 primary filters with a K. 530 or K. 510 barrier filter.

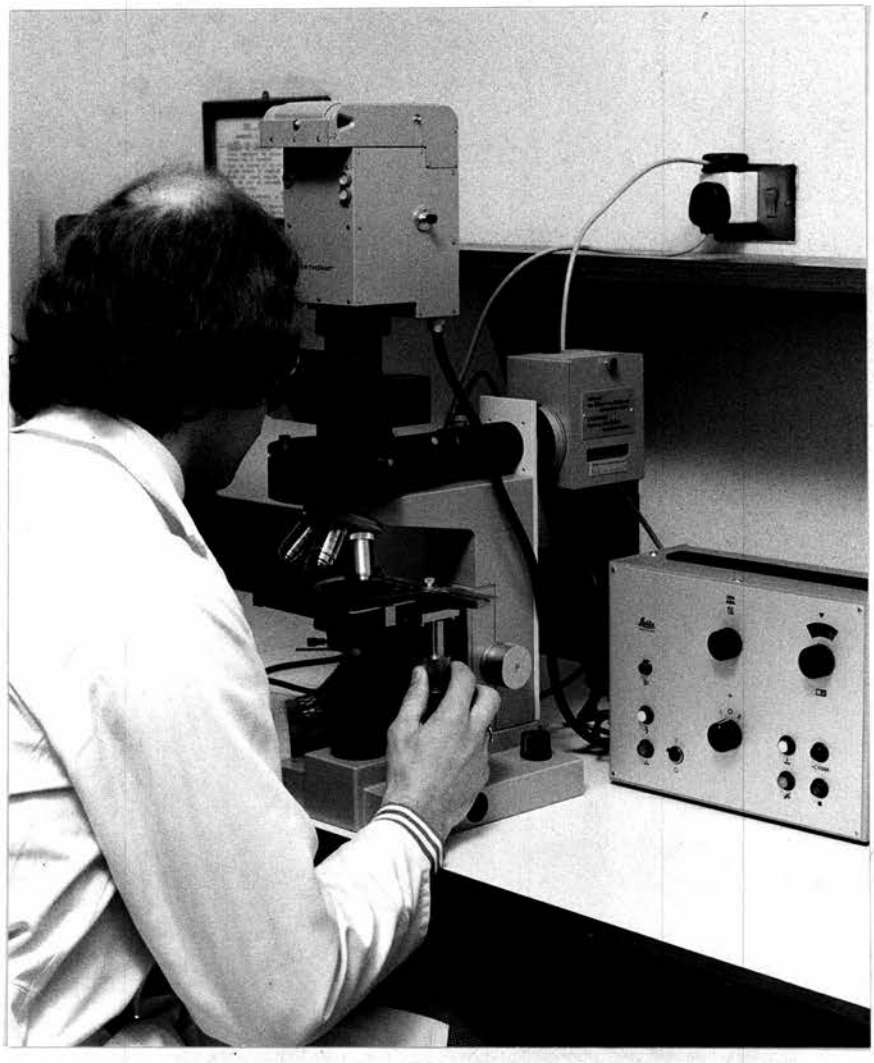
During the latter half of this study an incident of light illumination became available and this increased the resolution considerably.

4. Antisera

Commercial fluorescein-labelled antisera prepared in rabbits to human IgG, IgA, IgM, IgE, complement, (C_3 and C_4) and fibrinogen were obtained from Hoechst Pharmaceuticals. The specificity of the antisera was checked by immuno-electrophoresis (Fig. 45) and double diffusion in Agar gel (Fig. 46). The specificity of the fluorescein labelling was determined by viewing cellulose acetate strip electrophoresis under ultra-violet light. (Fig. 47).

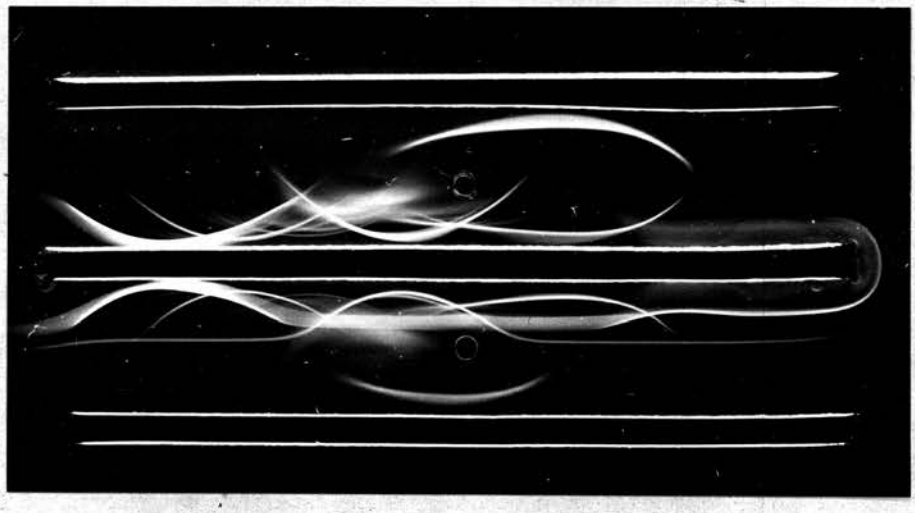
Initially the lyophilised antiserum was dissolved in 1 ml of distilled water and then absorbed with dried liver powder. It was found that absorbed and non-absorbed antisera gave similar results. In view of the fact that up to 20% of the antiserum could be lost

Figure 44



Leitz Incident Light Immunofluorescence Microscope

Figure 45

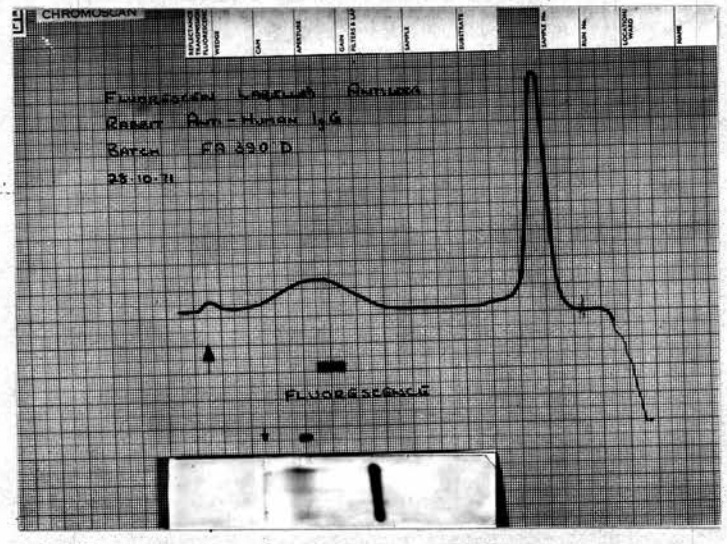


Immunoelectropheresis of antisera. Antiserum to IgG (Batch No. FA390D), to whole serum, and to IgA (Batch No. F389F) in the top, middle and lower troughs respectively, against normal serum in both wells. The IgG and IgA migrate to appropriate positions and precipitate with a single arc indicating a satisfactory serum.

Figure 46

Double diffusion in agar gel of antiserum to IgA, IgG, IgM, fibrin, complement (C_3) and whole serum against normal human serum. The immunoglobulin and complement precipitate as a single arc. Note the lack of any arc with antiserum to fibrin in view of the centre well containing serum.

Figure 47



Cellulose acetate strip electrophoresis of a fluorescein conjugated antiserum. The serum is run on cellular acetate, examined under ultraviolet light and the position of fluorescence noted. The strip is then stained and scanned and it can be seen that the fluorescence is localised to the immunoglobulin region indicating that the antiserum is correctly labelled.

during absorption this procedure was discontinued.

On each batch of antisera, dilution studies were carried out to determine the most appropriate concentration of antiserum. This was obtained by determining the concentration at which there was adequate immunofluorescence without an excessive background fluorescence. The lyophilised antiserum was stored at 4^{o} centigrade until required. Each ampoule containing the yophilised material was then re-suspended, using 1 ml of distilled water. Aliquots of 0.2 mls of this suspension were placed in small tubes and stored at $-20^{o}C$. Each week throughout the study a fresh tube of re-suspended antiserum was diluted to the appropriate concentration with phosphate buffered saline, stored at $4^{o}C$ and used as required.

5. Recording Results

Each biopsy was numbered and a record kept of the antisera batch numbers and the individual antisera concentration. The immunofluorescence data were recorded on a specially designed form (Fig. 41) which records the presence and distribution of the fluorescence throughout the specimen. In such a way it was possible to give a detailed analysis of the precise nature and location of material identified in this way. In the majority of cases positive results were photographed.

6. Photography

The immunofluorescence findings were photographed on Ektachrome daylight film using a Leitz Orthomat automatic camera. Exposed films were processed commercially and in many instances black and white prints were obtained from the colour transparencies.

IV RESULTS

1. DIFFUSE PROLIFERATIVE GLOMERULONEPHRITIS

Eighty biopsies have been performed in 73 patients with a diagnosis of diffuse proliferative glomerulonephritis. The results will be presented according to the initial clinical presentation except in those cases where treatment has been given and the biopsy is part of a follow-up study.

a. Acute Nephritis

In 14 patients the initial clinical presentation was as an acute nephritis. There were eight males and six females and their ages ranged from five years to sixty-nine years.

Hypertension was relatively common with a diastolic greater than 90 mm Hg in five of the fourteen patients. All had haematuria and proteinuria. The proteinuria varied from 0.26 g per 24 hours to 10 g per 24 hours. However, in only two patients did it exceed 3 g per 24 hours (case 109, 10.0 g per 24 hours; case 286, 7.0 g per 24 hours). In two patients the creatinine clearance at presentation was significantly reduced and it is interesting to note these are the two patients who progressed to chronic renal failure. A mild hypochromic anaemia was present in eight patients. The ASO titre was significantly elevated in four patients and in one there was a positive antinuclear factor although the L.E. cell preparation was repeatedly negative.

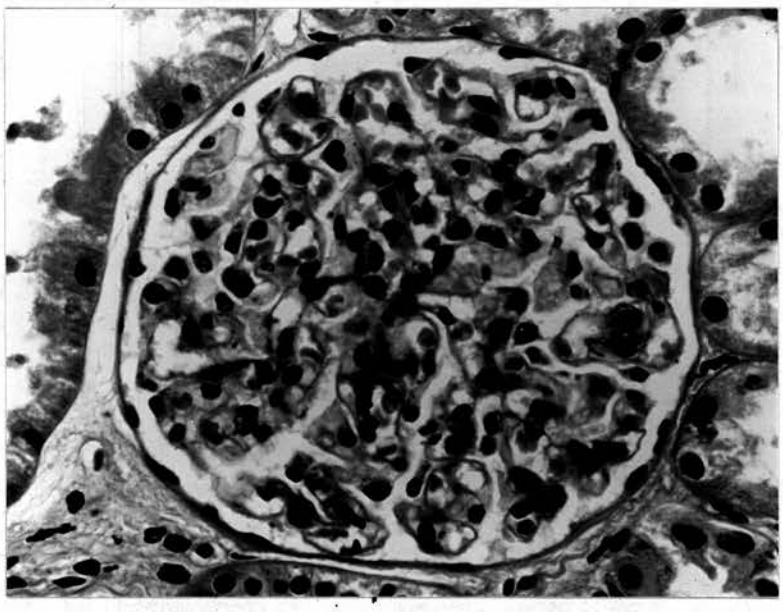
On histological examination one patient (case 271) had normal light microscopy and no positive immunofluorescence. However, this

patient had her episode of acute nephritis in 1959 and so the time interval between the clinical episode and the biopsy was 14 years, but she did, however, continue to have mild proteinuria and microscopic haematuria. In four patients there was a mild diffuse proliferative glomerulonephritis (Fig. 48), whilst in one the proliferation was of a moderate severity. An exudative diffuse proliferative glomerulonephritis was noted in seven patients (Fig. 49) whilst in one the histological features were of a progressive proliferative glomerulonephritis (II 4.b), (Table 5).

Immunofluorescence microscopy revealed the most common finding to be a granular deposition of IgG and fibrinogen along glomerular capillary walls (Fig. 50). This was present in eight patients. In addition small deposits of IgA were visualised within the capillary walls in seven patients and IgM in six. Complement (C_3) deposition was noted in seven patients. In the majority of patients the immunofluorescence was confined to glomerular capillary walls, but in three patients there were weak deposits of IgA and IgM in mesangial regions also. In three patients there was no positive immunofluorescence and in two cases the biopsy was performed a considerable time after the episode of acute nephritis, (case 208, 5 years and case 271, 14 years).

Three patients were treated with Indomethacin and one with Prednisone and the remaining ten had no specific therapy. At follow-up examination nine patients had returned to normal, but three continued to show proteinuria. In two cases there was a progressive deterioration to chronic renal failure. These two patients were considerably older than the majority; they did not have significant

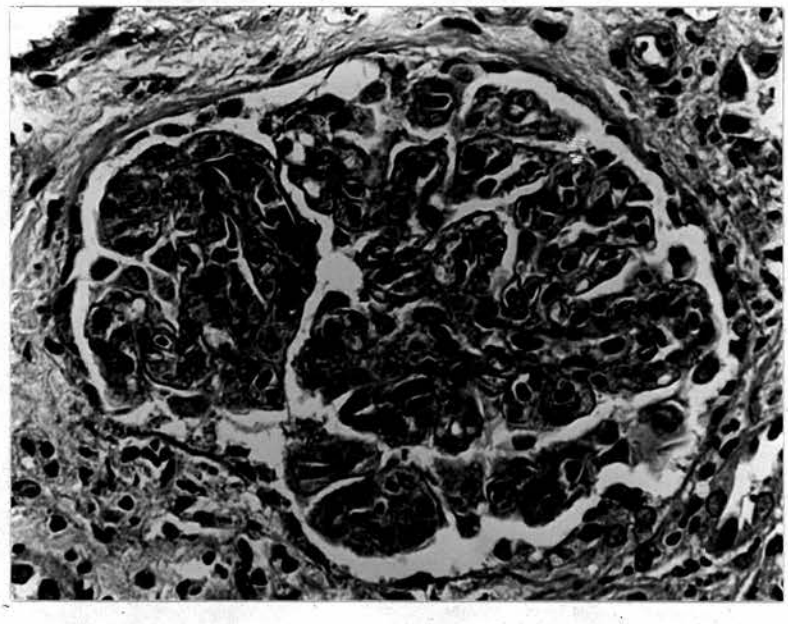
Figure 48



Mild diffuse proliferative glomerulonephritis

Case 212 H and E x 475

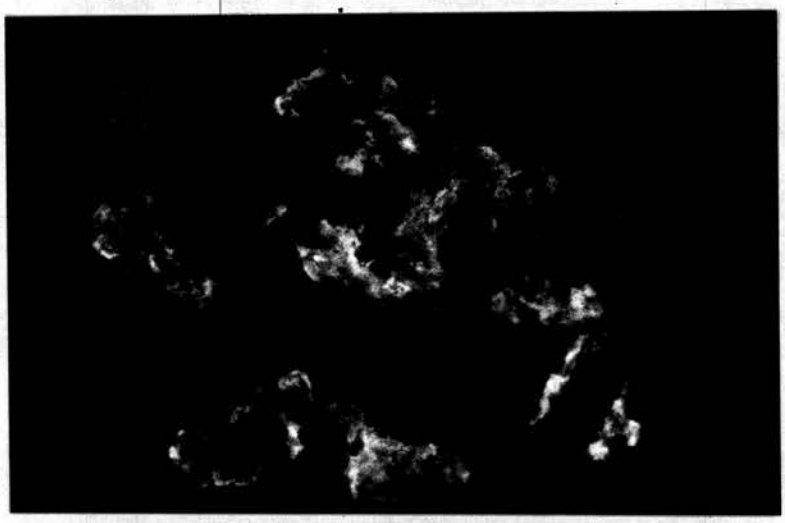
Figure 49



Exudative diffuse proliferative glomerulonephritis

Case 109 H and E x 425

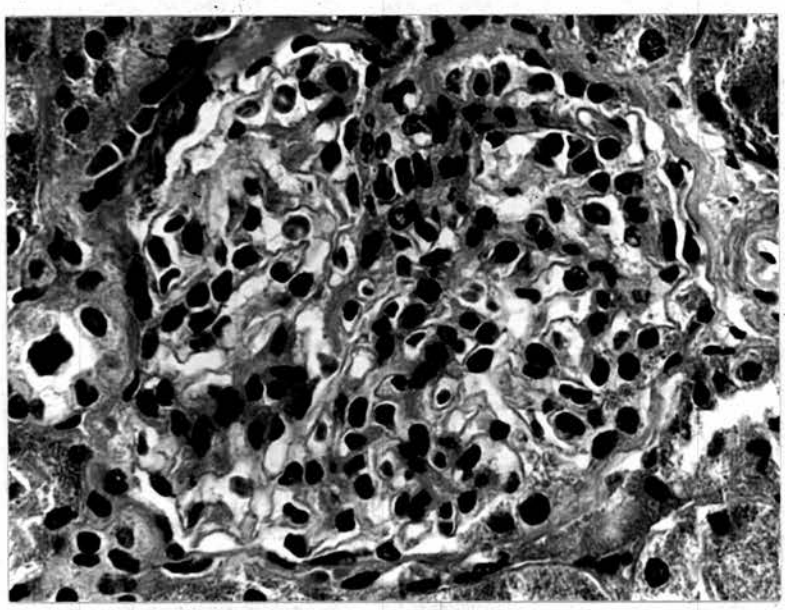
Figure 50



Granular deposition of fibrin/fibrinogen in glomerular capillary walls in a patient with mild diffuse proliferative glomerulonephritis.

Case 105 Fibrin/fibrinogen x 450

Figure 51



Mild diffuse proliferative glomerulonephritis in a patient with nephrotic syndrome.

Case 213 H and E x 550

hypertension but did show diminution of renal function when first examined. One patient (case 47) was a young child who presented with acute nephritis and eight years later continued to have proteinuria and haematuria. On further study it was revealed that his mother and cousin also had haematuria with little proteinuria. This suggests familial disease, but further details are not available.

b. Nephrotic Syndrome

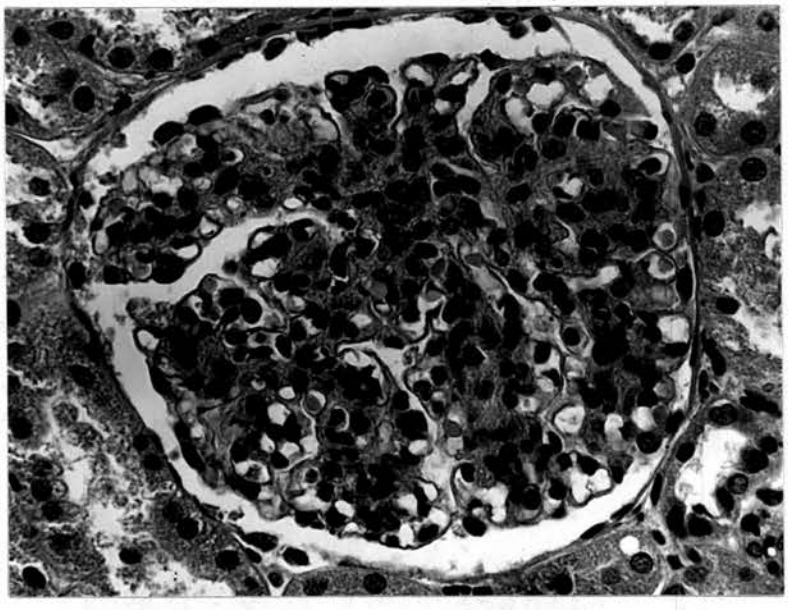
In 19 patients the initial presentation was as nephrotic syndrome. There were ten males and nine females and their ages ranged from 2 years to 70 years.

On clinical examination 10 of the 19 patients were hypertensive, with a diastolic pressure of 90 mm Hg or greater. In four there was haematuria and the proteinuria, at the time of biopsy, ranged from insignificant to 18 grams per 24 hours. Ten patients had protein excretion in excess of 4 grams per 24 hours. In only two patients was the creatinine clearance less than 50 mls per minute. Only one patient had a haemoglobin of less than 10 G/dl. In no patient was the ASO titre elevated or the ANF positive.

On histological examination there was a mild diffuse proliferative glomerulonephritis in 13 patients (Fig. 51). There was a moderate proliferation in four patients (Fig. 52) and progressive changes were noted in two. (Table 5).

On immunofluorescence microscopy fine granular deposition was noted in seven of twenty biopsies. The most common immunoglobulin was IgG, being present in five of the eight patients, while IgM was present in four. Fibrin deposition was noted in the glomerular

Figure 52



Moderate diffuse proliferative glomerulonephritis in a patient with nephrotic syndrome.

Case 132 H and E x 400

Figure 53

Fibrin/fibrinogen in a patient with moderate diffuse proliferative glomerulonephritis. The fibrin is deposited in a granular fashion in capillary walls and also to some extent in the mesangium.

Case 132 Fibrin/fibrinogen x 350

capillary walls of seven patients. (Fig. 53). In all patients with positive immunofluorescence there was considerable protein excretion, but the converse was not true. There did not appear to be any relationship between the presence of immunofluorescence and haematuria, hypertension or outcome.

At follow-up study eight patients continued to show abnormalities with respect to proteinuria and haematuria. Eight returned to normal and in one there was progression to renal failure and death. Tow patients were lost to adequate follow-up study.

c. Asymptomatic Proteinuria.

Fourteen patients presented with asymptomatic proteinuria. There were seven males and seven females whose ages ranged from 13 to 47 years.

On examination the diastolic blood pressure was 90 mm Hg or more in only one patient. Haematuria was detected in four patients. The creatinine clearance was greater than 50 mls per minute in all patients and the haemoglobin was less than 10 G/dl in only one patient. The protein excretion appeared to be minor, being less than 1 gram per 24 hours in seven patients and greater than 2 grams per 24 hours in only two (case 68, 2.6 g/24 hrs., case 196, 5.1 g/24 hrs.) The ASO titre was not elevated in any patient but a positive ANF was detected in two patients. On histological examination a mild diffuse proliferative glomerulonephritis was present in eleven patients. A moderate proliferation was noted in one patient whilst there were progressive changes in two patients (Table 5).

On immunofluorescence microscopy fibrin/fibrinogen deposition in glomerular capillary walls was the most common finding being present

in eight of the fourteen biopsies. IgM was present in six, IgG in five and IgA in three biopsies. These immunoglobulins were associated with complement deposition in six instances.

At follow-up examination six patients have returned to normal, whilst eight continue to have urinary abnormalities. None has shown evidence of progressive renal impairment.

Repeat biopsies were performed in three patients after therapy with Indomethacin.

d. Recurrent Haematuria

In thirteen patients the initial presentation was as recurrent haematuria. There were ten males and three females whose ages ranged between 5 and 35 years, with an average of 16.6 years.

Hypertension was not a feature of this group, being present only in one patient. Many patients had a long history of recurrent haematuria for periods varying between one and 23 years, with an average of about four years. Haematuria was present in all patients but proteinuria was not a marked feature, being less than 1 gram per 24 hours in ten patients. The remaining three had a daily protein excretion of 1.2, 1.5 and 3.2 G. The ASO titre was elevated in two while the ANF was negative in all patients.

On histological examination ten cases had a mild diffuse proliferative glomerulonephritis and in one the proliferation was of moderate severity. In two patients there were changes suggestive of a progressive process (Table 5).

Immunofluorescence was detected in only seven patients. This was in no way related to age, sex or severity of symptoms. Immunoglobulin deposition was found in five cases and in all it was

of small amount, present in granular deposition and confined to capillary walls.

At follow-up examination four patients had returned to normal, whilst eight continued to have episodes of recurrent haematuria. In one patient there was progressive deterioration in renal function until he required dialysis and subsequent transplantation. This patient showed on light microscopy changes of a progressive proliferative glomerulonephritis.

e. Hypertension

In three patients the initial presentation was as hypertension. There were two males and one female and their ages were between 19 and 51 years.

At initial presentation all showed diminution in creatinine clearance. They all had haematuria and proteinuria. In no case was the ASO titre elevated or the ANF positive.

On histological examination there was evidence of a progressive proliferative glomerulonephritis in two patients while in one it was mild and non-progressive, (Table 5). Immunofluorescence microscopy examination revealed considerable immunoglobulin, complement and fibrin deposition in the glomerular capillary walls of the two patients with progressive appearances by light microscopy. This was associated by immunoglobulin and complement deposition in mesangial areas. In the one patient with a mild proliferative glomerulonephritis the immunofluorescence examined was negative.

At follow-up examination the two patients with progressive histological appearances showed evidence of declining renal function, whilst the patient with mild proliferation continues to have proteinuria

and haematuria but has essentially unchanged renal function.

f. Asymptomatic Haematuria

Two patients presented with asymptomatic haematuria. These were both young being 12 and 9 years old respectively. The haematuria was microscopic but persistent. Proteinuria was insignificant. Renal function was normal and neither had hypertension.

On histological examination they both revealed a mild diffuse proliferative glomerulonephritis and by immunofluorescence examination no significant deposits were detected.

At follow-up examination they continued to show microscopic haematuria but no evidence of deteriorating renal function.

g. Repeat Biopsies

In thirteen patients with diffuse proliferative glomerulonephritis the biopsy was performed after a course of therapy. In eight instances this was after Indomethacin, in three cases after Prednisone and in two cases after a combination of Prednisone and Indomethacin. Eight of the patients were biopsied initially before the start of this study whilst in five cases the first and second biopsy were obtained during this study. In these five cases treatment with Indomethacin had been given between the first and second biopsy. In three of the patients the biopsy appearance was unchanged, in one the initial biopsy showed a mild diffuse proliferative glomerulonephritis and the subsequent showed progressive features, in the remaining case the initial biopsy was of moderate severity and subsequently this was of mild severity.

h. Follow-up Studies

At follow-up examination one to three years later patients were classified as being normal if all abnormal indices had returned to

normal. They were classified as improved if there was a significant improvement in any of the abnormal findings present at initial biopsy. If the abnormalities persisted they were classified as being unchanged and if there was a significant reduction in renal function they were classified as having deteriorated. Of the 73 patients with diffuse proliferative glomerulonephritis five were lost to adequate follow-up. The remaining 68 were studied to determine whether there were any prognostic indicators present at the time of initial biopsy which would give an indication as to the outcome of the illness.

There did not seem to be any relationship between the presence or absence of haematuria or the extent of proteinuria and prognosis. The age of the patient at time of initial presentation appeared to have some importance as far as prognosis was concerned; two of 29 patients under the age of 20 deteriorated and five of 39 patients over the age of 20 deteriorated. However, in considering patients over the age of 40, 4 of 18 patients deteriorated whilst only 2 of these 18 returned to normal. It is possible therefore that the absence of a clear cut relationship between age and prognosis is due to the small numbers in this study.

The immunofluorescence findings gave some indication as to prognosis. Of the 34 patients with no immunofluorescence to fibrin and the 33 patients with no immunofluorescence to any immunoglobulins only 2 showed any significant deterioration in their renal function. However, of the 11 patients with fibrin in the capillary walls and mesangium, 3 showed evidence of significant decline in renal function, and of the 26 patients with immunoglobulins in the capillary walls and mesangium or the mesangium alone, 5 showed decline in renal function.

(Table 6).

Hypertension appeared to be significant as far as prognosis was concerned. Of the twelve patients whose diastolic pressure was greater than 100 mm Hg at time of biopsy, 5 patients have deteriorated. This compares with only 2 of 45 patients in whom the diastolic pressure was less than 100 mm Hg. (Table 7).

The histological findings were also of importance. Of the 10 patients with a progressive type lesion (see II 5.b) 3 progressed to chronic renal failure. This compares with only 2 of 50 patients with mild or moderate non-progressive proliferative glomerulonephritis. Exudative lesions on histology are associated with a variable prognosis. Two of 7 patients deteriorated, whilst 3 returned to normal. (Table 8).

In diffuse proliferative glomerulonephritis those factors which seem to indicate a poor prognosis are:-

1. the presence of hypertension at initial presentation,
2. the age of the patient, the older patient probably having a poor prognosis,
3. the presence of a progressive lesion on histological examination, i.e. an irregular increase in mesangial matrix with or without small crescents,
4. The presence of immunoglobulins and/or fibrin in large amounts in the capillary walls and mesangial regions.

TABLE 5

DIFFUSE PROLIFERATIVE GLOMERULONEPHRITIS

Clinical presentation and histological findings in 64 patients not receiving any therapy at the time of biopsy.

Histology	Glomerulonephritis				
Presentation	normal	mild	moderate	exudative	progressive
acute nephritis	1	4	1	7	1
nephrotic syndrome		13	3		2
asymptomatic proteinuria		11	1		2
recurrent haematuria		10	1		2
hypertension		1			2
asymptomatic haematuria		2			

TABLE 6

DIFFUSE PROLIFERATIVE GLOMERULONEPHRITIS

Immunofluorescence findings and outcome.

Fibrin/Fibrinogen

	Nil	Walls	Walls & Mesangium	Mesangium
Normal	14	10	1	1
Improved	4	2	3	0
Same	14	7	4	1
Deteriorated	2	1	3	1

Immunoglobulins

Normal	14	6	6	0
Improved	5	1	3	0
Same	12	2	9	3
Deteriorated	2	0	3	2

TABLE 7

DIFFUSE PROLIFERATIVE GLOMERULONEPHRITIS

Blood pressure at initial presentation and outcome.

	Diastolic blood pressure	
	less than 100 mm Hg	greater than 100 mm Hg
Outcome		
Normal	20	3
Improved	7	2
Same	23	2
Deteriorated	2	5

TABLE 8

DIFFUSE PROLIFERATIVE GLOMERULONEPHRITIS

Histological appearances and outcome.

	Histology			
	mild	moderate	exudative	progressive
Normal	18	3	3	1
Improved	6	1	2	0
Same	19	1	0	6
Deteriorated	2	0	2	3

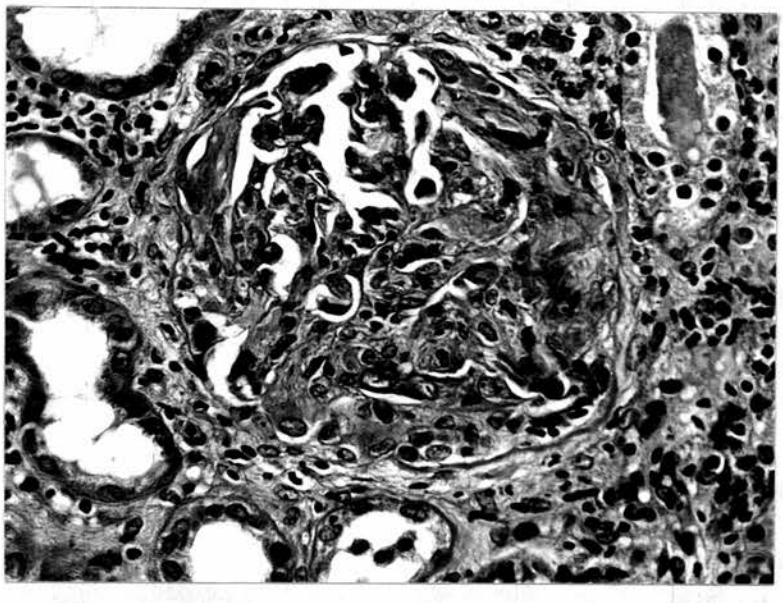
2. RAPIDLY PROGRESSIVE (CRESCENTIC) GLOMERULONEPHRITIS

Eight biopsies were studied from five patients with rapidly progressive proliferative glomerulonephritis. In all cases the biopsies were carried out as part of initial investigations of suspected underlying renal disease. In two instances the repeat biopsy was performed very shortly after the initial biopsy as it was felt inadequate tissue had been obtained on the first occasion.

This group consisted of three female and two male patients. The average age was 56 (34 to 69 years). All presented with acute oliguric renal failure. In four patients one of the presenting symptoms was breathlessness associated with upper respiratory tract infection, frontal headaches or joint pains. In the fifth patient the illness developed concurrently with a staphylococcal septicaemia. Hypertension was not a common feature in this group; the highest pressure obtained on admission was 175/90. Four patients showed evidence of congestive cardiac failure and four showed gross evidence of uraemia. All patients had haematuria. All had oliguria and required haemodialysis. In only one patient (case 231) was there an elevation of the ASO titre.

The histological features on light microscopy were the presence of large circumferential crescents in many glomeruli (Fig. 54). Within the glomeruli there was frequently endothelial cell swelling. Mesangial cell proliferation was not a feature, and in only one patient (case 231) was there any evidence of mild proliferation. Fibrin deposition was obvious in three cases and there was polymorph infiltration in four. The basement membrane appeared to have normal thickness. In all patients the tubules were dilated with evidence

Figure 54



Light microscopy in rapidly progressive (crescentic) glomerulonephritis showing a large crescent and a small residual glomerular tuft. The tubules show ischaemic changes.

Case 44 H and E x 320

Figure 55

Fibrin/fibrinogen deposition in a large circumferential crescent in a patient with rapidly progressive (crescentic) glomerulonephritis. Note the lack of immunofluorescence in the glomerular tuft.

Case 183 Fibrin/fibrinogen x 350

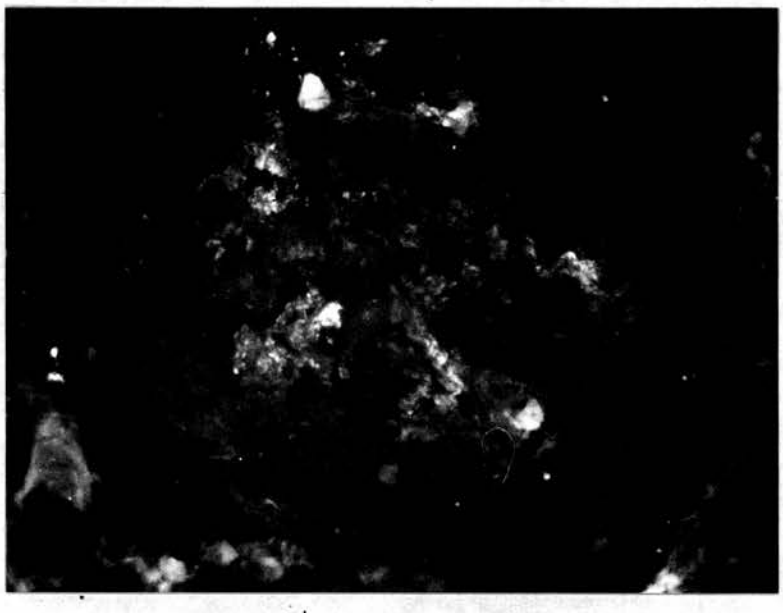
of tubular disruption. Throughout the interstitium there was frequently a diffuse infiltration with polymorphs, plasma cells and lymphocytes. In a few arterioles there was evidence of slight intimal thickening.

Immunofluorescence microscopy showed the most consistent finding to be the deposition of fibrin/fibrinogen within the large circumferential crescents (Fig. 55). In five of the seven biopsies there was weak granular deposition of IgG along glomerular capillary walls. This was associated with a granular deposition of IgM in one patient and C_3 deposition (Fig. 56) in two patients. In no case was there any linear deposition of immunoglobulin. In four cases there was a small amount of fibrin/fibrinogen within glomerular capillary walls. In no cases was there any evidence of immunofluorescence within mesangial regions (Table 9).

Four patients continued to require haemodialysis and three of these patients have subsequently died, one from broncho-pneumonia and two from electrolyte disorders. Only one patient (case 231) has shown any sign of improvement and some 15 months after her initial illness has a creatinine clearance of 92 mls per minute, is normotensive and has no evidence of renal disease.

In this series rapidly progressive proliferative glomerulonephritis has accounted for five of twenty-three patients presenting with acute renal failure. It accounts for some 3.4% (5 of 144) patients with primary glomerular disease.

Figure 56



Complement (C_3) deposition in a small amount in the glomerular capillary walls of a patient with rapidly progressive (crescentic) glomerulonephritis. Note the lack of fluorescence in the crescent.

Case 230 Complement (C_3) x 350

TABLE 9

RAPIDLY PROGRESSIVE PROLIFERATIVE GLOMERULONEPHRITIS

IMMUNOFLUORESCENCE FINDINGS

	CAPILLARY WALLS						MESANGIUM						CRESCENTS
	G	A	M	C_3	C_4	F	G	A	M	C_3	C_4	F	F
44	+	-	-	-	-	-	-	-	-	-	-	-	++
82	±	-	±	+	-	+	-	-	-	-	-	-	+
85	+	-	+	±	-	+	-	-	-	-	-	-	+
110	±	+	+	-	-	-	-	-	-	-	-	-	±
183	+	-	-	-	-	±	-	-	-	-	-	-	+
230	+	-	-	+	-	±	-	-	-	-	-	-	++
231	-	-	-	-	-	+	-	-	-	-	-	-	+
237	-	-	-	-	-	-	-	-	-	-	-	-	-

3. MEMBRANOUS GLOMERULONEPHRITIS

Seventeen biopsies were studied from sixteen patients with membranous glomerulonephritis. In ten cases the biopsy was carried out as part of the initial investigations. In seven instances biopsy was performed as part of a long term follow-up examination, and six of these patients had had therapy with either steroids or immunosuppressive agents. One patient had two biopsies within the time interval of this study.

The group consisted of thirteen male and three female patients. The mean age at onset of symptoms was 50 years and the range was from 22 years to 74 years. In fourteen instances the initial presenting feature was the nephrotic syndrome, whilst the remaining two patients presented with asymptomatic proteinuria. All patients had proteinuria and this varied in amount from 1 gram to 18 grams per 24 hours (selectivity 1.2 to 2.3). The creatinine clearance ranged from 16 mls per minute to 124 mls per minute. On initial clinical examination eight patients had diastolic hypertension (greater than 90 mm Hg) and this did not seem to be related either to the patient's age, extent of the proteinuria or to the creatinine clearance. In only three patients was haematuria detected at any time throughout their illness.

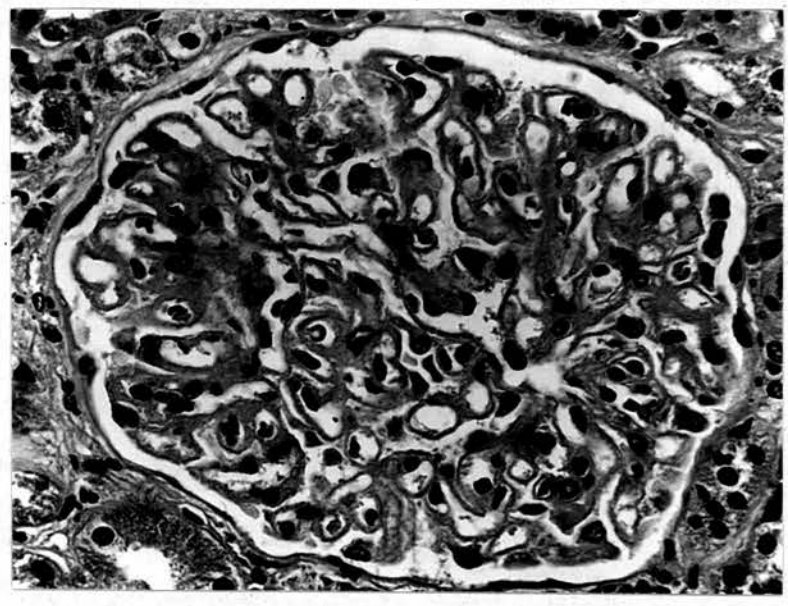
The main histological feature seen on light microscopy was a diffuse uniform increase in the thickness of the glomerular capillary walls (Fig. 57). Hyalinisation of glomeruli was common. In seven cases there was mild mesangial cell proliferation with some relative increase in mesangial matrix. Ten cases showed thickening of arteriolar walls of moderate degree, the presence of which did not

appear to bear any relationship to the blood pressure. Tubular atrophy with some interstitial fibrosis was seen in several cases. There was occasional intertubular oedema and inflammatory cell infiltrate.

Immunofluorescence examination revealed IgG in nine of the seventeen cases, IgA in three, IgM in three, C_3 in three and fibrin/fibrinogen in three: C_4 was demonstrated in two of seven cases. The distribution was in a granular fashion along glomerular capillary walls (Fig. 58). IgG always showed the brightest immunofluorescence, whereas fibrin/fibrinogen, although present with equal incidence, was much less prominent. The mesangial regions showed very little immunofluorescence. The immunofluorescence within the glomerular capillary walls could be related qualitatively to the time since the initial presenting symptoms. IgG appeared to be most prominent between ten and twenty-six months. In no instances was any immunofluorescence demonstrable after sixty-six months from initial presentation. Fibrin/fibrinogen appeared most prominent early in the illness and only small trace quantities were visible after ten months.

The patients were followed up from one to sixty-four months (average eighteen months). During this time two patients improved, seven remained static, three deteriorated, four died and one was lost to adequate follow-up. Chronic renal failure was the cause of death in three patients and pulmonary embolus in one patient. From this small study it did not appear as though the initial blood pressure, 24 hour urine protein, proteinuria selectivity or creatinine clearance bore any relationship to the outcome of the disease. It did, however,

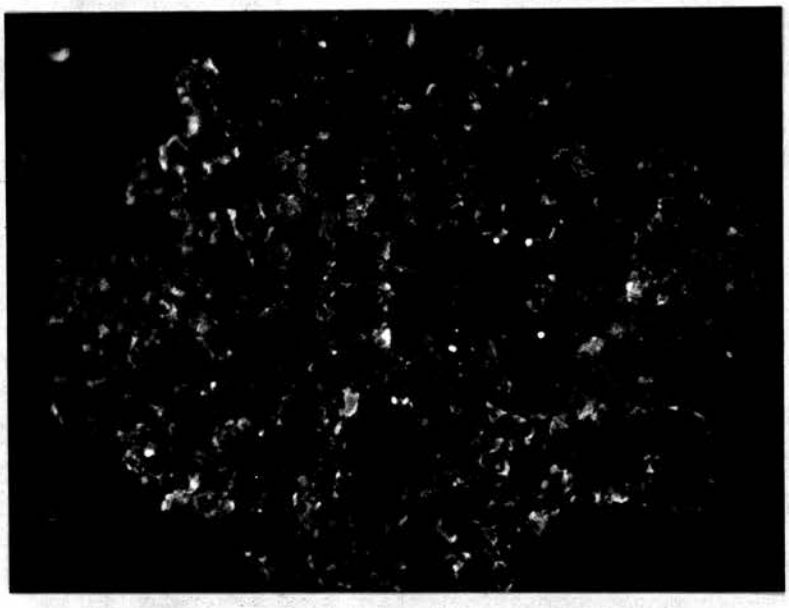
Figure 57



Diffuse uniform increase in the glomerular capillary walls of a patient with membranous glomerulonephritis.

Case 29 H and E x 425

Figure 58



Granular distribution of immunofluorescence to IgG on the subepithelial aspect of the glomerular capillary walls in a patient with membranous glomerulonephritis. Symptoms had been present for only ten months, (in addition see Fig. 13).

Case 228 IgG x 425

appear that the younger patients had a poorer prognosis than those in an older age group. Three of five patients whose symptoms developed prior to the age of forty have died, whereas only one of the twelve patients whose symptoms appeared after the age of forty is dead.

In this study membranous glomerulonephritis was the diagnosis in 14% of adults with primary renal disease (17 of 118 patients). However, it was the cause of 32% of adults presenting with nephrotic syndrome (15 of 47 patients).

TABLE 10

MEMBRANOUS GLOMERULONEPHRITIS

Glomerular capillary wall immunofluorescence and time since onset of symptoms

CASE	TIME	G	A	M	C_3	C_4	F
83	2	+	+	-	-		+
178	8	+	-	-	-		+
209	8	-	-	-	-		-
296	8	+	±	-	++	+	++
209	9	-	-	-	-		-
228	10	+	-	+	+	+	±
294	11	++	-	+	+	+	+
29	12	++	+	-			+
258	14	++	-	-	-	±	±
264	26	++	-	-	+	-	±
143	44	-	-	±	-		±
287	66	+	-	-	-	-	-
299	72	-	-	-	-	-	-
279	108	-	-	-	-	-	-
165	144	-	-	-	-	-	-
273	168	-	-	-	-	-	-
70	171	-	-	-	-	-	-

4. MESANGIOCAPILLARY GLOMERULONEPHRITIS

Fourteen biopsies were carried out in thirteen patients, nine males and four females, in whom a diagnosis of mesangiocapillary glomerulonephritis was made. Their age ranged from seven to sixty-one years (mean 31 years). In five patients the biopsy in this series was carried out as part of a follow-up study: two patients had been treated with Indomethacin and one patient with steroids.

Six patients presented with nephrotic syndrome, three with acute nephritis, two with asymptomatic proteinuria and two with hypertension. The time interval between initial presentation and biopsy varied from one month to fifteen years. In those patients in whom the biopsy was carried out as part of initial investigations the mean time since the onset of symptoms was three months. In patients in whom the biopsy was performed as part of a follow-up examination the time interval varied from six months to fifteen years.

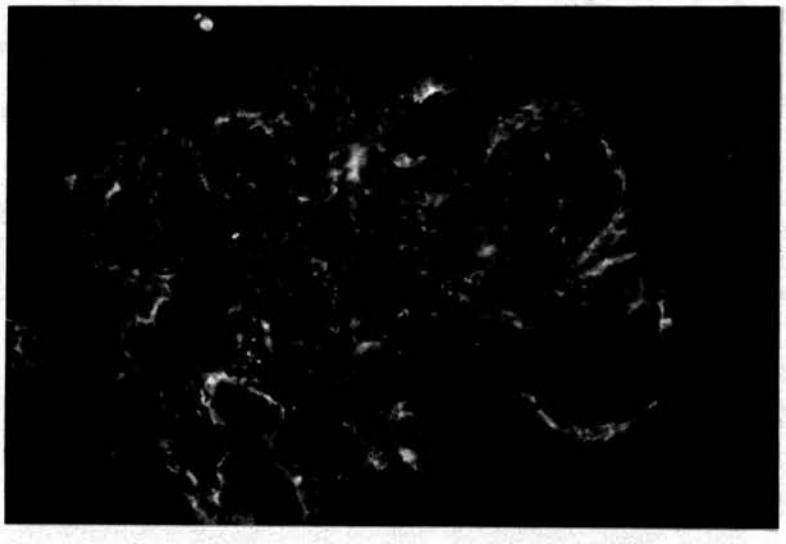
All patients exhibited proteinuria which varied in amount from 0.4 to 11.0 g/24 hours. The mean urinary protein excretion was 4.6 g/24 hours. Eight patients had significant haematuria at some time in their illness. The creatinine clearance varied between 8 and 147 mls/min and was below 80 mls/min in seven patients. A diastolic blood pressure greater than 100 mm Hg was recorded in seven of the thirteen patients. The plasma albumin was low in the majority of patients being greater than 3.3 g/l in only four patients. These four patients had the lowest urinary protein excretion and therefore it is likely that the plasma albumin only reflects the urinary protein loss.

Immunofluorescence examination revealed a granular deposition

of IgG distributed in the peripheral parts of glomerular capillary walls in six of the thirteen cases (Fig. 59). Complement (C_3) and fibrin/fibrinogen was present in six patients, IgM in five instances and IgA in three, with a similar granular distribution in the capillary walls. Towards the end of the study an antiserum to C_4 was obtained, and C_4 deposition was found in a granular fashion in the glomerular capillary walls in two of four cases examined (Fig. 60). The most striking feature was the lack of immunofluorescence within obviously enlarged mesangial regions. In only three cases was any immunofluorescence present and in each of these it was to a very small extent. The deposition of immunofluorescent material did not appear to be time dependent or in any way related to the mode of presentation of the illness. Bright immunofluorescence was present in one patient (case 226) who had biopsy proven mesangiocapillary glomerulonephritis seven years previously. The immunofluorescence did not bear any relationship to the ultrastructural type of mesangiocapillary glomerulonephritis, immunoglobulins being present with a similar distribution whether the patient had a sub-endothelial deposit type or a dense deposit type of glomerulonephritis. In addition the immunofluorescence appeared to bear no relationship to the outcome of the disease.

At follow-up examination one patient was shown to have improved as far as his renal function was concerned but proteinuria persisted. Seven patients remained unchanged and the remaining five patients deteriorated. The two patients who presented with asymptomatic proteinuria were among those who remained unchanged, one of six patients presenting as nephrotic syndrome deteriorated and required intermittent

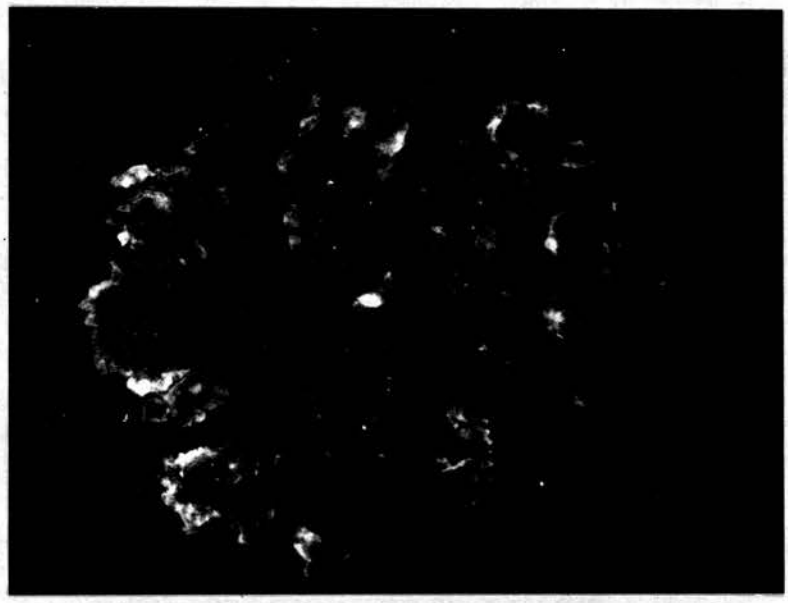
Figure 59



Immunofluorescence to IgG in the peripheral walls of glomerular capillaries in a patient with mesangiocapillary glomerulonephritis. Note the relative lack of fluorescence in the obviously enlarged mesangial regions. This patient had partial lipodystrophy.

Case 149 IgG x 350

Figure 60



Immunofluorescence to complement (C_4) with a similar distribution to the immunoglobulin deposition in Fig. 59.

Case 226 Complement (C_4) x 350

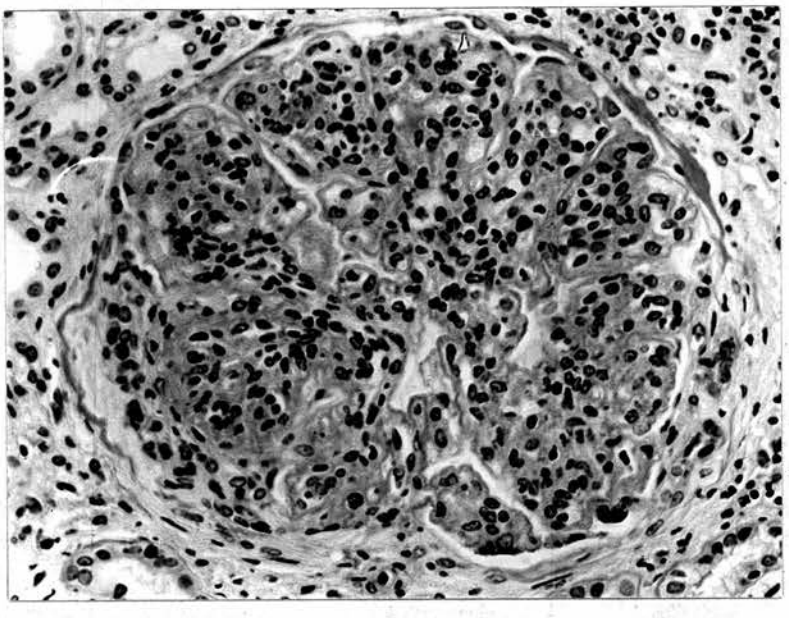
haemodialysis, two of three patients who presented as acute nephritis deteriorated, whilst one improved. One patient who presented as hypertension deteriorated and died from chronic renal failure and one remained unchanged. There were seven patients with the ultrastructural subendothelial deposit type disease; of these patients four either died or deteriorated to require intermittent haemodialysis. Five patients had dense deposit type disease and only one of these deteriorated to haemodialysis whilst four remained unchanged over the period of study. However, it is difficult to draw any conclusions from this in view of the small numbers in the study.

In summary, mesangiocapillary glomerulonephritis accounts for some 15% of patients with primary glomerular disease in this study. Clinical presentation is variable although proteinuria,haematuria and hypertension are frequent clinical findings. Immunofluorescence examination of biopsy material reveals a characteristic pattern of deposition of immunoglobulins, complement and fibrin within the peripheral walls of glomerular capillaries. The prognosis of patients with this condition is not good as approximately 50% have progressively deteriorating renal function.

Partial Lipodystrophy

Two biopsies have been obtained from patients with partial lipodystrophy. The first, a female of thirty-one at the time of biopsy, was found at a routine medical to have glycosuria. Some three years later she developed proteinuria and was found to have partial lipodystrophy. A biopsy at this time revealed mesangiocapillary glomerulonephritis, (Fig. 61). During a pregnancy her renal function deteriorated considerably at about thirty weeks

Figure 61



A mesangiocapillary glomerulonephritis of the "dense deposit" type in a patient with partial lipodystrophy.

Case 74 P.A.S. x 275

gestation. Following delivery she was treated with heparin infusions and her creatinine clearance rose satisfactorily to about 20 mls per minute. However, following discharge from hospital she was treated with an oral contraceptive, and her renal function once again deteriorated, and she subsequently required to be treated with intermittent haemodialysis. The second (case 149) was a female aged seventeen who was noticed to have partial lipodystrophy from the age of five. Her renal function deteriorated steadily and she too had to be started on intermittent haemodialysis. The light microscopy findings in these two patients revealed a mesangiocapillary glomerulonephritis. Immunofluorescence examination showed considerable immunoglobulin, complement and fibrin deposition, most prominent in the peripheral capillary walls, but there was also a small amount of IgM and fibrin in the enlarged mesangial regions. These appearances were identical to the immunofluorescence in patients with mesangiocapillary glomerulonephritis, without associated partial lipodystrophy.

5. FOCAL PROLIFERATIVE GLOMERULONEPHRITIS

Twelve biopsies were carried out in eleven patients with focal proliferative glomerulonephritis. There were eight males and three females in this group and the mean age was twenty-nine (range 3 years to 71 years). One patient had had a previous biopsy and in this study was biopsied to determine the effects of therapy with Indomethacin. One patient (case 94) had a follow-up biopsy during this study.

The clinical presentation was nephrotic syndrome in four patients, asymptomatic proteinuria in two patients, acute nephritis in two patients, hypertension in two patients and asymptomatic haematuria in one patient. On initial examination only three of the eleven patients were hypertensive (i.e. a diastolic pressure greater than 90 mm Hg). All patients had proteinuria, with a mean value of 2.9 ± 1.8 g/24 hours. Three patients exhibited haematuria but in two of these it was microscopic. Creatinine clearance was normal in nine patients and diminished in only two. The E.S.R. was elevated in six patients but this did not bear any relationship to either the presentation or outcome. In no patient was the A.S.O. titre elevated and in all the A.N.F. was negative. In five patients in whom serum complement was established serum C_3 fell within the normal range.

Immunofluorescence microscopy revealed that the most remarkable finding was that in only one patient (case 101) was there evidence that the deposition of immunofluorescence had a focal distribution. In all other instances the immunoglobulin, complement and fibrin had a fairly even distribution in the capillary walls of all gomeruli. The most common finding was a granular deposition of IgM in capillary

walls (Fig. 62). This was present in seven of eleven cases. In addition to this there was a granular deposition of IgG in glomerular capillary walls in four cases, of IgA in two cases, of complement (C_3) in four cases and of fibrin/fibrinogen in five cases. In mesangial regions the most common finding was a deposition of IgM but this was present in only three of the eleven patients. Other immunoglobulins were present to only a minimal extent although fibrin/fibrinogen was seen in two patients.

On light microscopy the glomeruli showed evidence of minor endothelial cell swelling associated with some enlargement of the mesangial regions and an increase in mesangial cells. The mesangial cell proliferation was not present in all glomeruli and appeared to occupy only part of the glomerular tuft, giving a segmental appearance. In two of the eleven cases there was evidence of necrosis in the foci of proliferation (Fig. 63). In a few biopsies there was a moderate increase in polymorph infiltration within the glomeruli. Capillary walls were of normal thickness and there was no occlusion to the capillary lumen. The interstitial tissue revealed no specific abnormality and there was no evidence of oedema or fibrosis. Tubules appeared normal. In one biopsy there was fibrinoid necrosis in an arteriole.

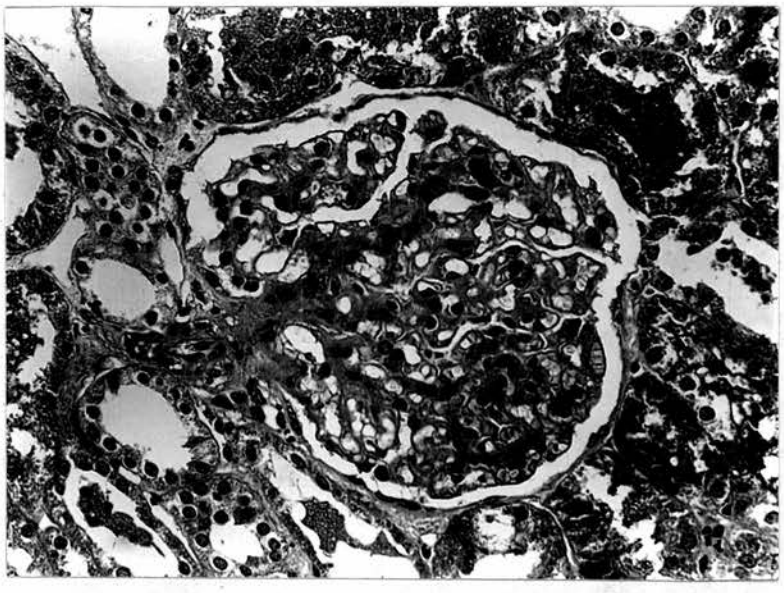
Unfortunately in this group of patients three were lost to adequate follow-up. Two patients died, one from chronic renal failure and one from a viral infection contracted whilst on steroids. Three patients required no specific therapy, whilst four patients were treated symptomatically for either their peripheral oedema or hypertension. One patient was treated with a combination of Prednisone

Figure 62

Immunofluorescence to IgM in focal proliferative glomerulonephritis. Although there is some segmental accentuation of deposition immunofluorescence is present throughout the glomerulus.

Case 95 IgM x 350

Figure 63



This is a glomerulus from a case of focal proliferative glomerulonephritis showing a segmental proliferation.

Case 247 H and E x 300

and Cyclophosphamide and appeared to show some reduction in proteinuria. One patient was treated with steroids alone and two were treated with Indomethacin. There was no clear evidence from this small study that therapy made any difference to the underlying lesion.

The most striking finding in this group of patients, as already mentioned, was that while light microscopy showed clear evidence of focal proliferation of mesangial cells, by immunofluorescence microscopy the immune material was seen to be evenly distributed throughout all glomeruli without any evidence of a focal accentuation of deposition.

In this study focal proliferative glomerulonephritis accounts for some 8% of patients with primary glomerular disease. Clinical presentation is extremely variable and from the clinical history there does not appear to be any clue as to the underlying aetiology. The immunofluorescence was remarkable in as much as it was diffuse, whereas the histological changes appeared focal. The prognosis was not good, three patients showing progressing deterioration in renal function. Neither the mode of presentation nor the immunofluorescence findings provided any indication of the prognosis.

6. MESANGIAL IgG/IgA DISEASE

Two patients with mesangial IgG/IgA disease were identified during this study. They were both males, one aged twenty-five and one aged six.

The first (case 43) was found to have proteinuria at a routine medical examination. Subsequently he developed an upper respiratory tract infection and concurrent haematuria. The second was found to have haematuria unrelated to any other illness. There was no significant past history or family history in either case. On examination both were moderately hypertensive (blood pressure at initial investigation: case 43, 160/98; case 255, 115/90). Both exhibited marked haematuria, but only case 43 had significant proteinuria (3 G/24 hrs.). Renal function as estimated by creatinine clearance was normal in both patients. The ESR was not elevated, the ASO titre was not raised and the ANF was negative in both patients.

By light microscopy both showed a moderate increase in mesangial matrix with a slight increase in mesangial cells. No other significant lesion was detected and in particular no evidence of progressive disease or hypertensive change.

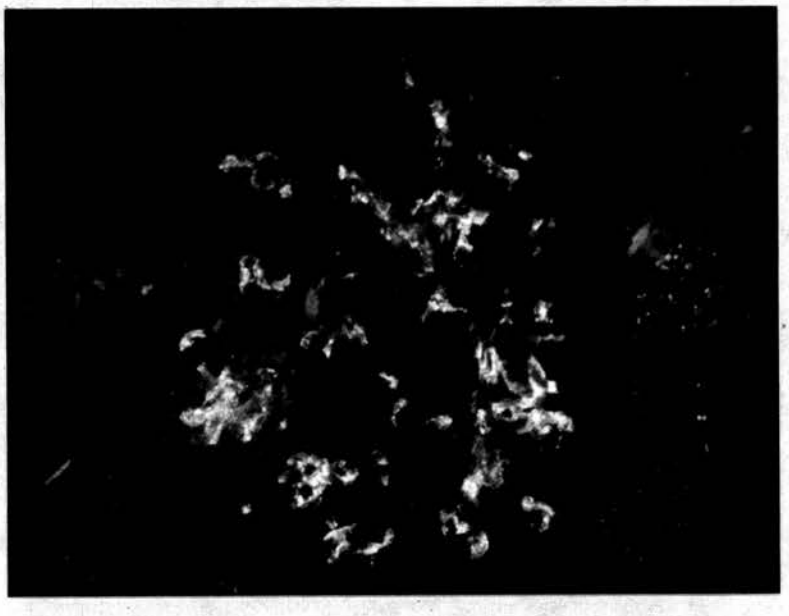
On immunofluorescence microscopy both showed extensive granular deposition of IgG and IgA within mesangial regions (Fig. 64). This was associated with some complement (C_3) deposition with a similar distribution. In addition both cases showed minor deposition of fibrin/fibrinogen within glomerular capillary walls. Only one case was examined for C_4 and this was negative.

Unfortunately one patient was lost to follow-up. The second patient, however, continued to show recurrent haematuria frequently

associated with upper respiratory tract infections.

In this study mesangial IgG/IgA disease was detected in only two patients, i.e. approximately 1% of patients with primary glomerular disease. Both were males and both showed the syndrome of recurrent haematuria associated with upper respiratory tract infection. Light microscopy reveals only minor changes within the glomerulus and immunofluorescence microscopy is required to establish the diagnosis. It is not possible from this limited number to give any indication as to prognosis.

Figure 64



Immunofluorescence to IgA in mesangial IgG/IgA disease. The fluorescence is confined to the mesangium. (See also Figs. 3 and 22).

Case 225 IgA x 375

7. MINIMAL LESION GLOMERULONEPHRITIS

Twenty-four biopsies were carried out in twenty patients diagnosed as having minimal lesion glomerulonephritis. The mean age of these patients was 15.4 years (range 1 to 70 years). There were thirteen males and seven females in this group. There were only two patients over twenty (case 11, aged 70 and case 256, aged 50).

All patients presented with the nephrotic syndrome. One developed clinical features approximately one month after measles immunisation. In two patients the nephrotic syndrome followed within seven days of an upper respiratory tract infection and in one other patient the nephrotic syndrome developed after a week's illness which consisted of malaise, cough and abdominal pain. On examination there was perceptive nerve deafness in one patient. Proteinuria varied from 2.8 grams per 24 hours to 26 grams per 24 hours, and the uncorrected creatinine clearance between 15 mls per minute and 126 mls per minute. No patient had haematuria at any time, and no patient was hypertensive at initial presentation.

On light microscopy the only findings were a minor increase in mesangial cells associated with some slight increase of mesangial matrix in fourteen of twenty-four biopsies. In one further biopsy there was some increase in mesangial cells but no associated increase in mesangial matrix. There was no evidence of glomerular hyalinisation, endothelial cell proliferation, basement membrane thickening or polymorph infiltration. In the interstitium there was no fibrosis or cellular infiltration and the tubules and blood vessels appeared normal.

On immunofluorescence examination a small amount of fibrin/

fibrinogen was demonstrated in glomerular capillary walls in eight of the twenty-four biopsies (Fig. 65). In one patient a small amount of fibrin/fibrinogen was present in glomerular capillary walls in both biopsies taken two weeks apart. In one (case 53) there was a small amount of IgM in glomerular capillary walls. Three cases were examined by specific IgE anti-serum and they were all negative.

Follow-up examination was carried out over a period of twelve to thirty-six months (average 25 months). All patients were treated with Prednisone and in seven patients who had frequent relapses on withdrawal of Prednisone a combination of Prednisone and Cyclophosphamide. All patients had normal renal function and at follow up all were in remission. However in view of the fact that many of these patients show frequent relapses it is not possible to state that all patients are cured.

In this study, minimal lesion glomerulonephritis accounts for some 36% of all children with primary glomerular disease. It is interesting to note, however, that it accounts for only some two-thirds of children presenting with the nephrotic syndrome. It is a disease characterised by a nephrotic presentation, absence of haematuria and of hypertension and response to treatment with Prednisone. Some are dependent upon steroids but it may require a combination of Prednisone and Cyclophosphamide for total remission. It occurs predominantly in children but can occur at any age. The prognosis is good and none of the patients in this group have progressed to renal failure.

Figure 65

A small amount of fibrin/fibrinogen in a few glomerular capillary walls of a patient with minimal lesion glomerulonephritis.

Case 53 Fibrin/fibrinogen x 450

8. FOCAL GLOMERULOSCLEROSIS

In this study five patients have been diagnosed as having focal glomerulosclerosis. There were three males and two females and their ages ranged from 2 to 71. Four were biopsied prior to any specific therapy but one had been on steroids for 24 days prior to renal biopsy.

Two patients presented with nephrotic syndrome and two with asymptomatic proteinuria whereas one presented with hypertension. Four patients had haematuria, and all had proteinuria of a moderate degree, ranging from 2 grams to 3.8 grams per 24 hours. Four patients were hypertensive, with a diastolic pressure greater than 90 mm Hg.

On light mocroscopy the most significant finding was that approximately 50% of glomeruli were completely hyalinised. In the remaining glomeruli many showed irregular enlarged mesangial regions, in some sufficiently marked to obliterate the lumina of several capillaries, producing patchy hyalinisation. In none was there any significant polymorph infiltration but one (case 89) showed small crescents, and one (case 205) showed capsular adhesions.

On immunofluorescence microscopy two were negative. In the remaining three patients one showed only a small amount of fibrin in glomerular capillary walls, one showed IgG and fibrin in walls and mesangium and IgM and C_3 in the mesangium, and the remaining patient had IgG, IgA and fibrin in the walls and IgG, IgA, IgM, C_4 and fibrin in the mesangium.

At follow-up examination one patient returned to normal (case 284). This patient was aged 2 and was the only patient who was normotensive at presentation. He was treated with steroids and his proteinuria remitted completely. Of the remaining four patients three still have

significant proteinuria and are moderately hypertensive. One patient (case 206) who presented with hypertension and on initial investigation had a diminished creatinine clearance, progressed to intermittent haemodialysis. It is not possible from this small number to give any indication of prognostic features in this condition.

9. HENOCH-SCHONLEIN PURPURA

Twelve biopsies were carried out in eleven patients with a diagnosis of Henoch-Schonlein purpura. There were seven males and four females in this group and their ages ranged from five to sixty-three years, though only three patients were over seventeen years of age.

At initial presentation all patients had haematuria and a purpuric rash, with the typical distribution in the lower limbs. In addition to these findings six patients presented with joint pain which was typically in the small joints. Six patients also complained of abdominal pain which was characteristically colicky in nature and poorly localised. Three patients had peripheral oedema and on clinical investigation each of these patients had a low plasma albumin. Three patients on presentation had positive faecal occult blood. No patient was hypertensive. Proteinuria was present to a variable extent: it was insignificant in amount in five patients, whilst in the other six it ranged from 0.4 to 7.0 g/24 hrs. The creatinine clearance was considered to be within the normal range in seven of the eleven patients. In four patients it was diminished, being 69 ml/minute in case 40, 13 ml/minute in case 92, 56 ml/minute in case 266 and 54 ml/minute in case 222. At follow-up examination five patients had become normal, one had only mild proteinuria and one had occasional haematuria. Three patients had evidence of declining renal function and these had had diminished renal function on initial investigation. Two progressed to intermittent haemodialysis and one died from a pulmonary embolus. Further laboratory investigation revealed an elevated ASO titre in one patient (case 9).

The anti-nuclear factor was negative in all patients tested, and serum complement (C_3) was normal. The ESR was normal in seven patients and was elevated in the three patients with diminished renal function. Platelet counts were within the normal range in all patients. IgG and IgA concentrations were normal, but in three of five cases the serum IgM was significantly diminished.

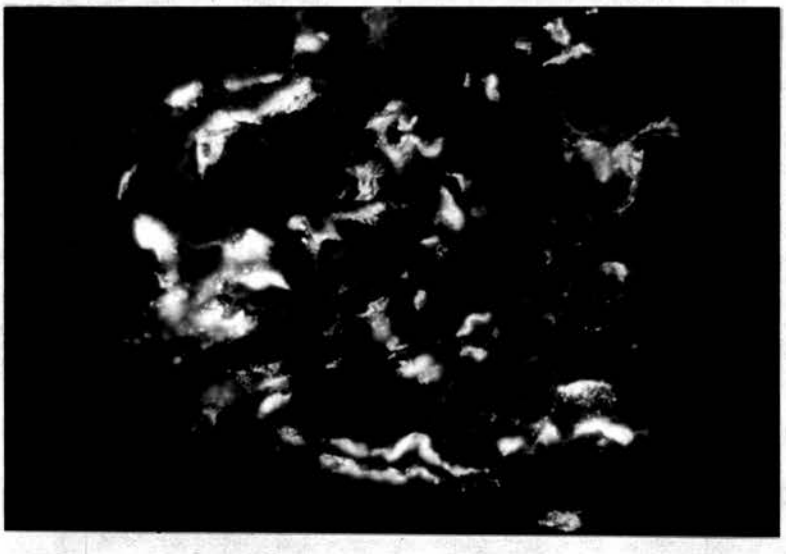
On immunofluorescence examination the most common finding was the presence of IgG, IgA and fibrin in glomerular capillary walls (Fig. 66). They had a fine granular distribution which in places looked almost linear. In two of ten cases IgM was present, in small amounts, and complement (C_3) was present in three patients. Mesangial deposition of material was not common, small amounts of IgG being present in three cases and small amounts of IgA in two. Fibrin/fibrinogen was demonstrable within the crescents in two patients (cases 92 and 266).

On light microscopy the most common finding was a minor proliferation of mesangial and endothelial cells. In three cases this proliferation appeared to have a focal distribution. In one case the endothelial and mesangial cell proliferation was associated with a minor increase in mesangial matrix. Polymorph infiltration was present in only three cases and these three also showed crescent formation. It is also interesting to note that it is these three cases which showed diminished renal function and either progressed to intermittent haemodialysis or died. One other patient (case 222) had crescent formation but there was no polymorph infiltration. Although renal function was diminished at presentation the patient improved, though he did not return to normal.

In this study Henoch-Schonlein purpura was diagnosed in eleven patients. In seven cases the illness regressed completely, in two patients renal function progressively deteriorated and the patients subsequently required intermittent haemodialysis. One patient with declining renal function developed a pulmonary embolus and died. From this limited survey it would appear that the following are indicators of poor prognosis:-

1. diminished renal function on presentation,
2. elevation of the ESR,
3. crescents by light microscopy,
4. significant polymorph infiltration on light microscopy.

Figure 66



Immunofluorescence to IgA in Henoch Schonlein disease. The deposition of material is in the capillary walls although there is an accentuation towards the hilum of the glomerulus.

Case 147 IgA x 350

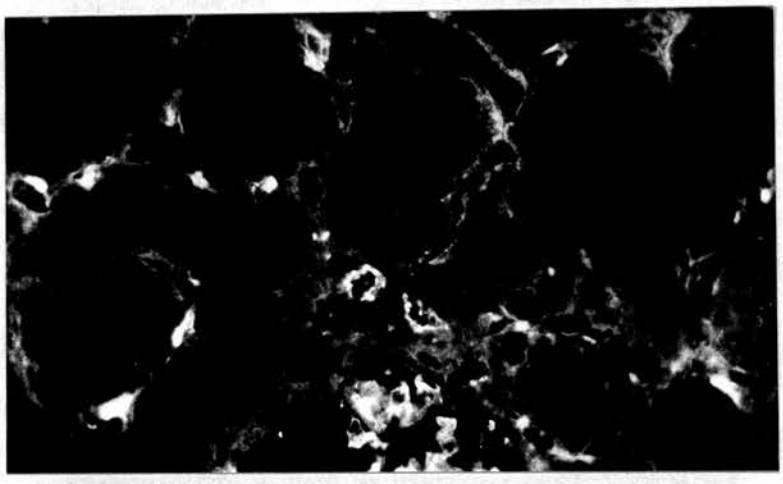
10. ACUTE TUBULAR NECROSIS

Eleven biopsies were carried out in nine patients with acute tubular necrosis. All patients had acute oliguric renal failure consequent upon some specific incident, septic abortion, Staphylococcal septicaemia, acute pancreatitis, sodium chlorate poisoning, paracetamol overdose, leptospirosis, anti-partum haemorrhage, septicaemia from an unknown site, and multiple myeloma. The age of the patients ranged from twenty-five to fifty-nine years and there were five females and four males.

All patients had acute oliguric renal failure and seven of the nine required intermittent haemodialysis. One patient died prior to the institution of dialysis and one patient established an adequate diuresis without the need for haemodialysis. Six patients returned to normal renal function, two died and one progressed to intermittent haemodialysis, (case 290, multiple myeloma).

Immunofluorescence examination revealed that the most common finding was a diffuse staining of the oedematous intertubular region with fibrin/fibrinogen (Fig. 67). In only four cases fibrin/fibrinogen was demonstrable within glomerular capillary walls. In two patients there was, in addition, deposition of IgM and C_3 within the glomerular capillary walls (case 141, leptospirosis, case 233 anti-partum haemorrhage). In three patients there were interstitial cells containing immunoglobulins, and presumably these are the plasma cells frequently seen on light microscopy (Fig. 68). In no case was there any deposition of IgG or IgA in capillary walls and no immunofluorescence was visible within any mesangial regions.

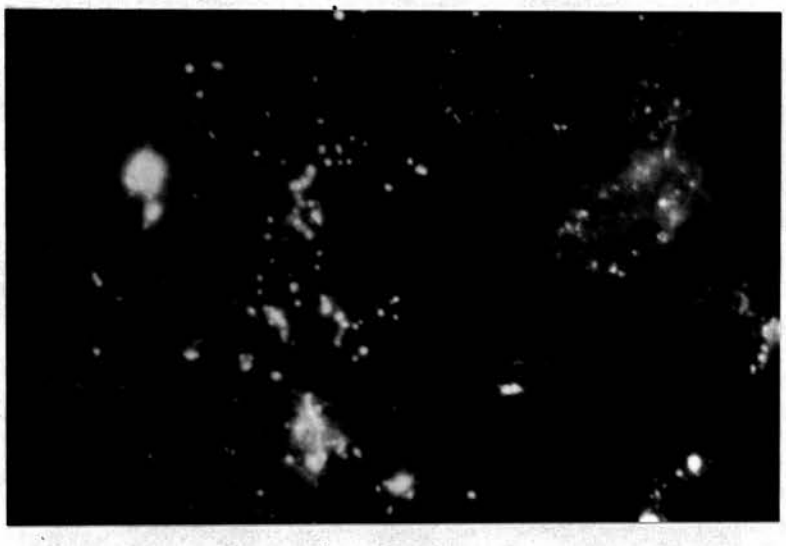
Figure 67



Deposition of fibrin/fibrinogen in the interstitium in a patient with acute tubular necrosis.

Case 129 Fibrin/fibrinogen x 300

Figure 68



IgM containing cells in the interstitium of a patient with acute tubular necrosis.

Case 69 IgM x 250

11. DISSEMINATED INTRAVASCULAR COAGULATION

Nine biopsies were carried out in seven patients in whom a diagnosis of disseminated intravascular coagulation had been made. There were four females and three males in this group, and there ages ranged from two to sixty-five years.

Patients in this group seldom presented with symptoms referable to the urinary tract, although four patients presented with acute renal failure. In all patients there was a short preceding illness. In three this amounted to an episode of nausea, vomiting and diarrhoea suggestive of a gastro-intestinal infection; in two patients there was evidence of preceding chest infection and one had pneumococcal meningitis. The remaining patient presented with a brief history of breathlessness and anorexia and she was subsequently found to be anaemic and uraemic (case 3).

Initial investigations in these patients revealed a thrombocytopenia with platelet counts ranging from less than 10,000 to 136,000. This was associated with anaemia and a raised plasma haemoglobin and reduced plasma haptoglobin in most patients. In the two youngest patients, both aged two, renal function was not seriously impaired, but in four adults there was oliguria. In the remaining patient (case 124) death occurred from fulminating intra-pulmonary haemorrhage within twelve hours of hospital admission and before renal function could be estimated, although the blood urea was known to be moderately elevated.

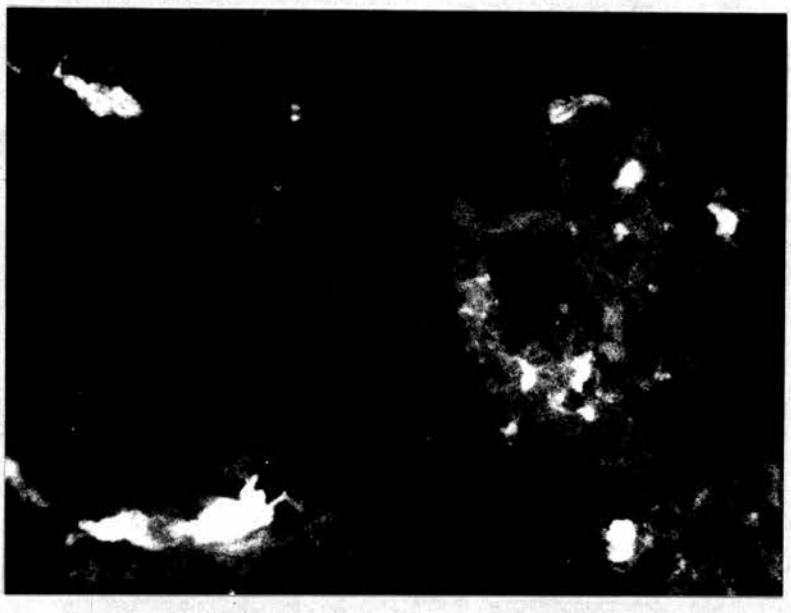
Three patients required treatment with intermittent haemodialysis. Two returned to normal renal function and one died from cardiac failure. One patient was treated with systemic heparinization, but died two

weeks later from a cerebrovascular accident. One child (case 77) was treated symptomatically and recovered normal renal function, two other patients died, one from fulminating intra-pulmonary haemorrhage and the other from pneumococcal meningitis.

The immunofluorescence findings in these patients revealed a diffuse granular deposition of fibrin in glomerular capillary walls in five of seven patients (Fig. 69). This was associated with obvious fibrin deposition within arterioles in four of the patients (Fig. 70). In one patient (case 122) there was evidence of IgM deposition in glomerular capillary walls and this was associated with a very weak deposition of IgG, IgA and complement (C_3) (Fig. 71). In only one other patient (case 3) was there any immunofluorescence to complement in glomerular capillary walls. Two other patients (case 32 and case 124) showed weak immunofluorescence to IgM in glomerular capillary walls (Fig. 72). In the one patient who had three biopsies, the most consistent finding was the demonstration of fibrin in glomerular capillary walls. IgG was demonstrated within glomerular capillary walls in the first two biopsies in this patient but not in the third. IgA was demonstrable in all three biopsies and IgM in two of the three biopsies. In this case all the immunoglobulins were present in small amounts.

On light microscopy glomeruli show minor proliferation of mesangial cells, although in some instances there was also endothelial cell swelling. No significant polymorph infiltration was seen in any case. The glomerular capillary walls in some instances showed irregular thickening of the basement membrane by deposition of MSB-positive material in a sub-endothelial situation. In a few

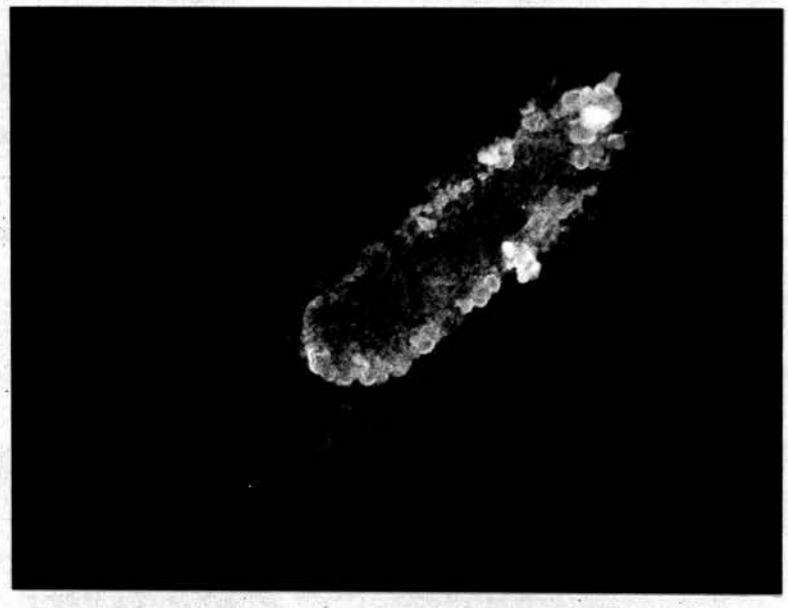
Figure 69



Fibrin/fibrinogen in the glomerular capillary lumina and also two peritubular capillaries in a patient with disseminated intravascular coagulation.

Case 124 Fibrin/fibrinogen x 450

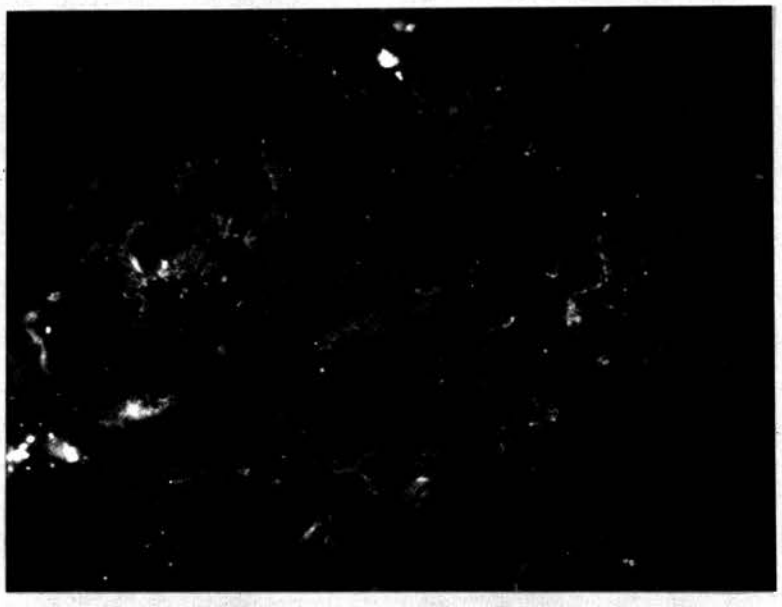
Figure 70



A fibrin plug occluding the lumen of a vessel in a patient with disseminated intravascular coagulation.

Case 130 Fibrin/fibrinogen x 500

Figure 71



A small amount of complement (C_3) in the glomerular capillary wall of a patient with disseminated intravascular coagulation.

Case 122 Complement (C_3) x 300

Figure 72

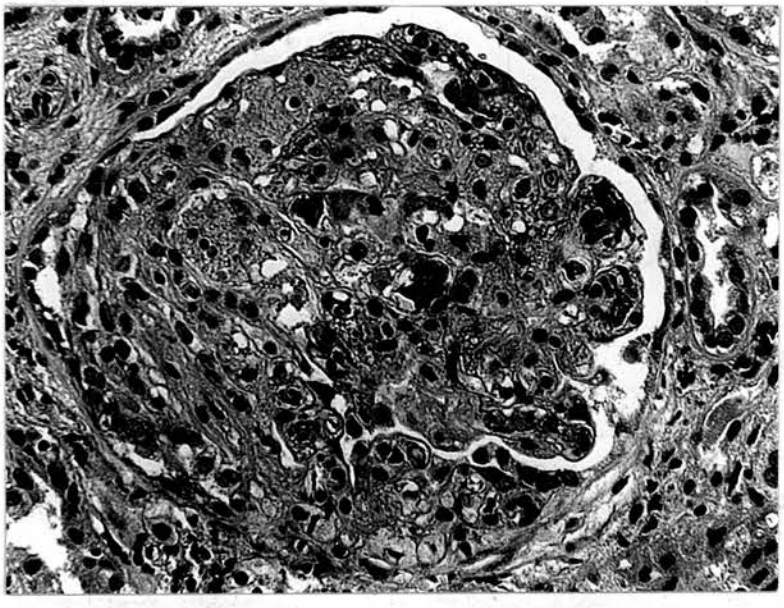
IgM deposition in glomerular capillary walls and mesangial regions of a patient with disseminated intravascular coagulation.

Case 122 IgM x 350

instances this was seen to occlude the capillary lumen (Fig. 73). In only one patient was there any significant interstitial oedema, although in another patient there was evidence of acute tubular necrosis. Fibrosis was noted in the interstitium of two patients and there was an interstitial infiltrate with chronic inflammatory cells in one of these patients. The arterioles frequently showed fibrinoid necrosis and evidence of fibrin deposition in their lumen. In some patients this was sufficient to cause occlusion thrombosis (Fig. 74).

In this group of seven patients there were four deaths, one from cardiac failure, one from a cerebrovascular accident, one from intrapulmonary haemorrhage and one from meningococcal meningitis. Three patients recovered normal renal function and are well, with no renal abnormality. Of the three patients who recovered normal renal function, none was treated with anticoagulants, but two required haemodialysis for acute renal failure.

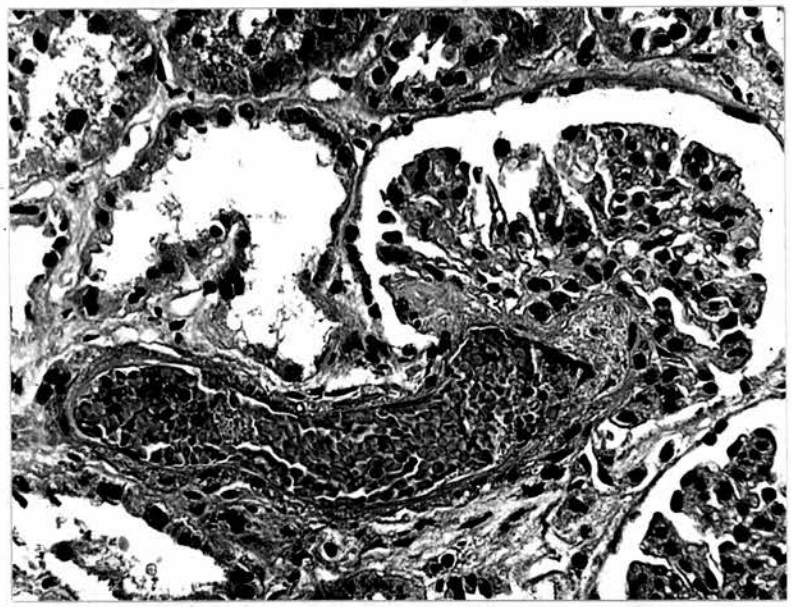
Figure 73



Fibrin thrombi occluding several capillary lumina in a patient with disseminated intravascular coagulation.

Case 122 M.S.B. x 300

Figure 74



A thrombi in the afferent arteriole of a glomerulus in a patient with disseminated intravascular coagulation.

Case 124 H and E x 325

12. GOODPASTURE'S SYNDROME

One patient with a presumptive diagnosis of Goodpasture's Syndrome is included in this study. This was a twenty-three year old man who presented with anorexia, vomiting and dyspnoea for less than a month's duration. On clinical exmination he had acute oliguric renal failure with haematuria and proteinuria associated with a moderate hypertension of 195/100. He had several episodes of frank haemoptysis.

On investigation his blood urea was 450 mg% with a haemoglobin of 6.6 G/dl and a platelet count of 170,000 per cubic mm. His ANF, ASO titre, and Australia antigen were all negative.

On immunofluorescence microscopy there was a fine, probably linear, deposition of IgG, localised to the capillary walls of tufts compressed by crescents. In addition there was occasionally a very faint granular deposition of complement (C_3) and fibrinogen in a similar position. Some fibrin was present in several crescents. There was no immunofluorescence to IgA or IgM within the glomeruli. The interstitium contained several cells in which IgG could be demonstrated.

On light microscopy in those glomeruli in which some capillary structure was evident there was no mesangial cell proliferation. Special staining showed a little fibrin in some still visible glomerular capillaries. Epithelial crescents were present in some glomeruli but many had been converted to balls of hyaline tissue. The overall appearances were the terminal stages of a progressive "crescentic" glomerulonephritis.

This man progressed to end stage renal failure. He was treated

with high dose corticosteroids, and whilst this improved his haemoptysis it did not improve his renal failure. Bilateral nephrectomy was performed and he had no further haemoptysis. After bilateral nephrectomy his steroids were rapidly tailed off to zero. He has been subsequently maintained on intermittent haemodialysis and more recently transplantation.

Goodpasture's Syndrome was diagnosed on the linear staining of IgG on the glomerular basement membrane in a patient who had acute oliguric renal failure and haemoptysis. Specific tests for anti-glomerular basement membrane antibody were not available but the response to bilateral nephrectomy is characteristic of this syndrome.

13. SYSTEMIC LUPUS ERYTHEMATOSUS

Ten biopsies were obtained from ten patients with systemic lupus erythematosus. In eight patients the diagnosis had been reached prior to renal biopsy for clinical presentation and investigative findings. In two patients the diagnosis of lupus erythematosus was suspected clinically but had not been confirmed. The eight patients known to have disseminated lupus erythematosus were on steroid therapy at the time of biopsy, while the two patients suspected of disseminated lupus erythematosus were on no specific therapy.

All patients in this group were female and their age at initial presentation varied from 14 to 46 (average 27.8 years). At the time of biopsy their age range was 21 to 58 years (average 35.9 years). The initial presenting feature was a typical skin rash in four patients, arthropathy in two patients, Raynaud's phenomenon in one patient, nephrotic syndrome in one patient, proteinuria and haematuria in one patient and bruising due to thrombocytopenia in one patient. All patients except one had haematuria and eight of the ten patients exhibited proteinuria (range 0.9 grams to 10.7 grams per 24 hours) in spite of steroid therapy. The creatinine clearance in this group of patients ranged from 23 mls per minute to 97 mls per minute, although one patient was anuric at presentation and had developed acute renal failure from an acute exacerbation of her lupus nephritis. In seven of the ten patients the diastolic blood pressure was greater than 95 mm of mercury.

On light microscopy all patients showed evidence of a proliferative glomerulonephritis, and no patients exhibited the "membranous" type of lupus nephritis. In all patients the proliferation was of mesangial

cells, although two patients in addition had significant endothelial cell proliferation. The proliferation appeared to be focal in three patients and diffuse in the others. A 'wire loop' (i.e. MSB-positive) thickening of the basement membrane was present in three cases. Focal necrosis was present in the glomeruli of two patients and in one there was recent capillary thrombosis. There were epithelial crescents in two patients. Lesions, consisting mainly of a diffuse thickening, were present in arteriolar walls in five patients, and in two of these patients there was obvious fibrinoid change in the vessel wall.

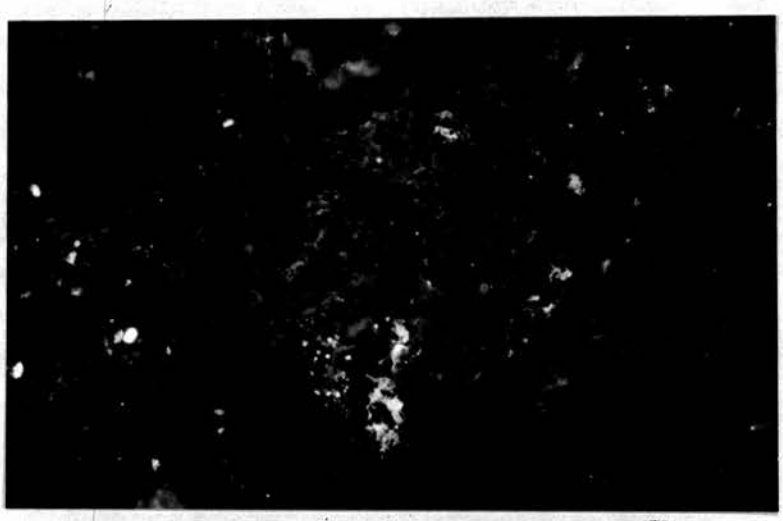
The immunofluorescence findings are shown in Table 11. The most common finding was that of fibrin deposition in glomerular capillary walls (Fig. 75). This was most frequently associated with IgG deposition, although IgM was also present in three cases and IgA in one case. Complement was not a significant feature except in one patient. Mesangial deposition of material was not common, although again the most frequent finding was that of fibrin. In a few patients immunoglobulins were visible within the mesangial regions and in each of these cases immunoglobulins were also present within capillary walls. In some cases IgG could be demonstrated on the nuclei of cells (Fig. 76).

During the follow-up period in this group of patients six showed no evidence of declining renal function. In three patients,however, renal function deteriorated to a significant extent. The one patient who presented with an exacerbation severe enough to cause acute renal failure did not regain any function and subsequently died from electrolyte imbalance. One other patient, case 67, died from a chest

infection which developed consequent to bilateral pneumothorax.

In this study disseminated lupus erythematotus was confined entirely to females. The age range was considerable, with a mean age of 27.8 years at initial presentation. Only two patients in this group died, one from acute renal failure and one from complications of systemic disease.

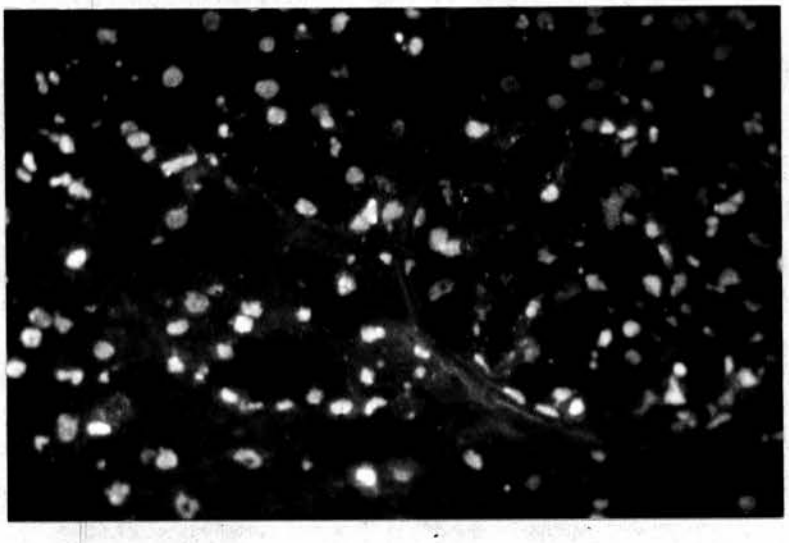
Figure 75



Fibrin/fibrinogen in a glomerulus, particularly at the hilum, of a patient with systemic lupus erythematosus.

Case 67 Fibrin/fibrinogen x 300

Figure 76



Nuclear localisation of IgG in a patient with systemic lupus erythematosus.

Case 180 IgG x 300

TABLE 11

SYSTEMIC LUPUS ERYTHEMATOSUS

IMMUNOFLUORESCENCE FINDINGS

	CAPILLARY WALLS						MESANGIUM					
	G	A	M	C_3	C_4	F	G	A	M	C_3	C_4	F
21	+	-	-	-	/	+	++	+	+	-	/	+
64	+	++	+	++	/	+	-	+	-	+	/	±
67	±	-	-	±	/	+	-	-	-	-	/	±
113	-	-	±	-	/	±	-	-	-	-	/	-
180	+	-	-	-	/	+	-	-	-	-	/	+
198	+	-	+	-	/	+	±	-	-	-	/	±
224	-	-	-	-	-	+	-	-	-	-	-	-
249	-	-	-	-	-	-	-	-	-	-	-	-
283	-	-	-	-	-	-	-	-	-	-	-	-
298	+	-	-	-	-	-	-	-	-	-	-	-

14. POLYARTERITIS

Thirteen biopsies were carried out in twelve patients with polyarteritis, (seven males and four females). In ten patients the diagnosis of polyarteritis was suspected on clinical presentation and initial biochemical findings. One patient was known to have polyarteritis and one patient presented with acute renal failure of unknown aetiology. The average age was 55 years (range 35 to 65 years). The time interval between the onset of symptoms and renal biopsy was nine months (range one month to three years). At the time of biopsy only two patients (case 8 and case 156) were receiving steroid therapy.

In six patients the initial clinical presentation was respiratory, in four the main symptom was breathlessness, whilst in two it was pleuritic chest pain. Two patients at presentation had acute renal failure. Two patients had joint pains involving the hands. One patient had symptoms of central nervous system involvement with some arteritic process and the remaining patient presented with haematuria, weight loss, and anorexia.

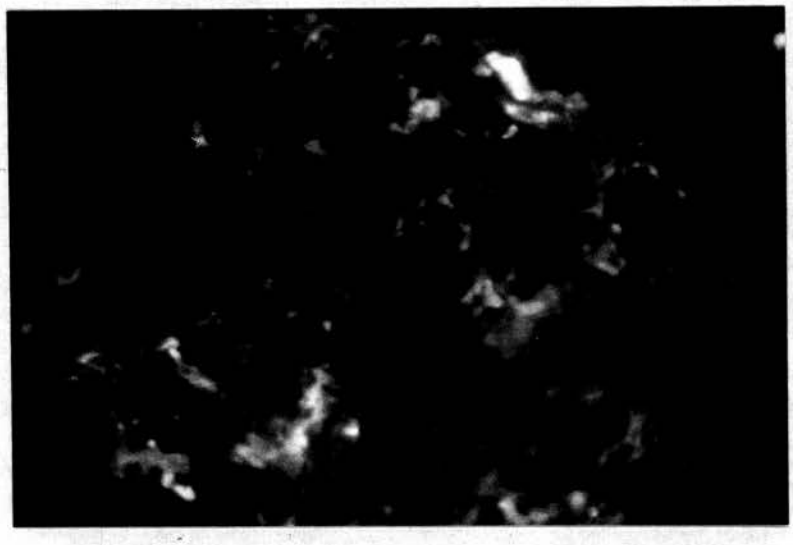
Proteinuria was not a common finding in these patients, being present in only five of the eleven. It ranged from 1.3 grams to 12.5 grams per 24 hours. Two patients, however, presented with oliguria. Six of eight patients tested had significant haematuria in the form of red cell casts. Renal function was considerably depressed, the creatinine clearance being less than 50 mls per minute in nine of the eleven patients. Hypertension was not a common feature, all patients except one having a diastolic pressure of less than 100 mm Hg.

On light microscopy the most prominent finding was a proliferative glomerulonephritis. In five patients this was a mild diffuse proliferation of mesangial cells. In three patients the proliferation appeared to have a focal distribution, whilst in two there was a focal necrotising glomerulonephritis. In one patient all glomeruli in the sections examined were hyalinised. In four patients there were circumferential crescents. In six patients there was obvious fibrinoid necrosis in arterioles. In two patients there was obvious blood vessel wall thickening, whilst in three there was perivascular cuffing with inflammatory cells.

On immunofluorescence microscopy the most common finding was fibrin deposition within glomerular capillary walls (Table 12). This was distributed in a granular fashion, and was present in eight of the thirteen biopsies studied. (Fig. 77). The next most common finding was complement (C_3) deposition in a similar distribution. In four patients IgG was present, two showed IgA and in two IgM was present in capillary walls. There was little in the way of immunofluorescence within the mesangial regions. In the arterioles complement (C_3) was visible in one case (Fig. 78) (case 276) and IgM was present in the arteriolar wall in one case (case 157).

Over the period of follow-up six patients remained essentially unchanged. All patients except case 161 were treated with steroids; the initial daily dose varying from 30 mg. to 80 mg. Three patients died and one patient went on to intermittent haemodialysis. In only two patients was there any evidence of improvement. The patients who deteriorated presented initially with very poor renal function or anuria and it would appear that this gives the best indication of the

Figure 77



Fibrin/fibrinogen in the glomerular capillary walls and occasional mesangial region of a patient with microscopic polyarteritis.

Case 54 Fibrin/fibrinogen x 350

Figure 78

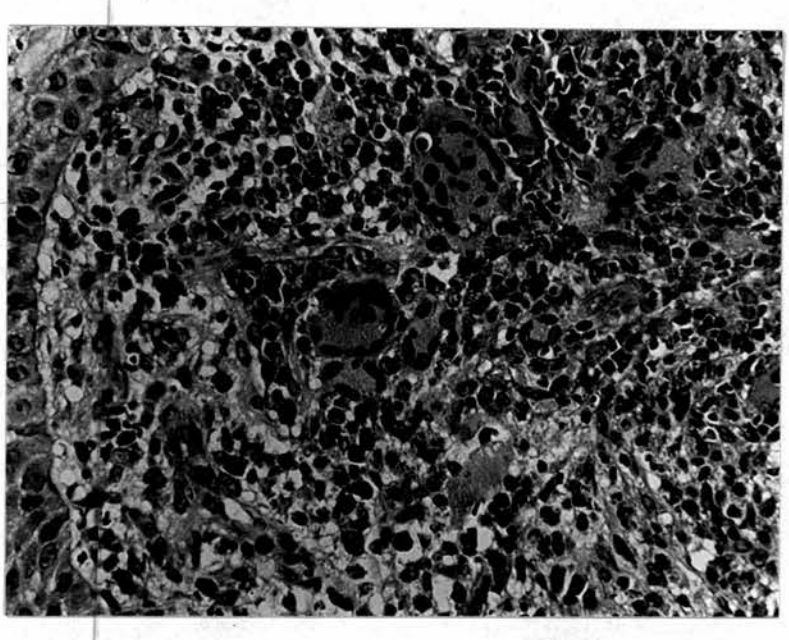
Complement (C_3) in glomerular capillary walls and also blood vessel walls of a patient with microscopic polyarteritis.

Case 276 Complement (C_3) x 300

outcome in any individual patient.

One patient with a diagnosis of Wegener's granuloma has been examined. This patient presented with nasal stuffiness, epistaxis, headache and deafness in his left ear. On examination he was found to have perforation of the cartilagenous part of the nasal septum and this perforation appeared to have a granular margin. A biopsy of this revealed changes typical of Wegener's granuloma (Fig. 79). He was treated with steroids and progressed satisfactorily until some seven months later when he developed a 'flu like illness consisting of polyarthritis, nystagmus and double vision. At this time he was found to have significant proteinuria and a creatinine clearance that was reduced to sixty-one mls per minute. In view of this a renal biopsy was performed. On light microscopy there was a focal and moderate proliferation of mesangial cells with some polymorph infiltration. Several glomeruli had crescents (Fig. 80). In many glomeruli there was increased mesangial matrix with some obvious narrowing of the associated capillary lumina. The interstitial tissue was focally infiltrated with inflammatory cells but no granulomas were seen. Several arterioles had thickened walls but there was no necrosis or inflammation in these walls. Immunofluorescence examination revealed a diffuse deposition of IgA and IgM in the capillary walls, but there was no other specific immunofluorescence. Following this 'flu like illness his steroid therapy was increased and Azathioprine was introduced. He progressed satisfactorily, although his renal function remains slightly depressed.

Figure 79



Biopsy of a midline granuloma of a patient with Wegeners Granulomatosis. There are several giant cells visible.

Case 139 H and E x 350

Figure 80

The renal biopsy from a patient with Wegener's Granulomatosus showing a fibrinoid lesion in the glomerulus, associated with a crescent.

Case 139 H and E x 275

TABLE 12

POLYARTERITIS

IMMUNOFLUORESCENCE FINDINGS

	GLOMERULAR CAPILLARY WALLS						MESANGIUM					
	G	A	M	C_3	C_4	F	G	A	M	C_3	C_4	F
8	-	-	-	-	-	±	-	-	-	-	-	-
54	+	-	-	-	-	+	+	-	+	-	-	+
98	-	-	-	-	-	-	-	-	-	-	-	-
108	+	+	-	+	-	+	-	+	+	+	-	-
118	±	-	+	+	-	-	-	-	-	-	-	-
156	-	-	-	-	-	±	-	-	-	-	-	-
* 157	+	-	±	+	-	±	-	-	+	-	-	-
161	-	-	-	-	-	±	-	-	-	-	-	-
204	-	-	-	-	-	+	+	-	-	+	-	+
254	-	-	-	-	-	+	-	-	-	-	-	-
276	-	+	-	+	-	-	-	-	-	-	-	-
282	-	-	-	-	-	-	-	-	-	-	-	-

* repeat biopsy of 118

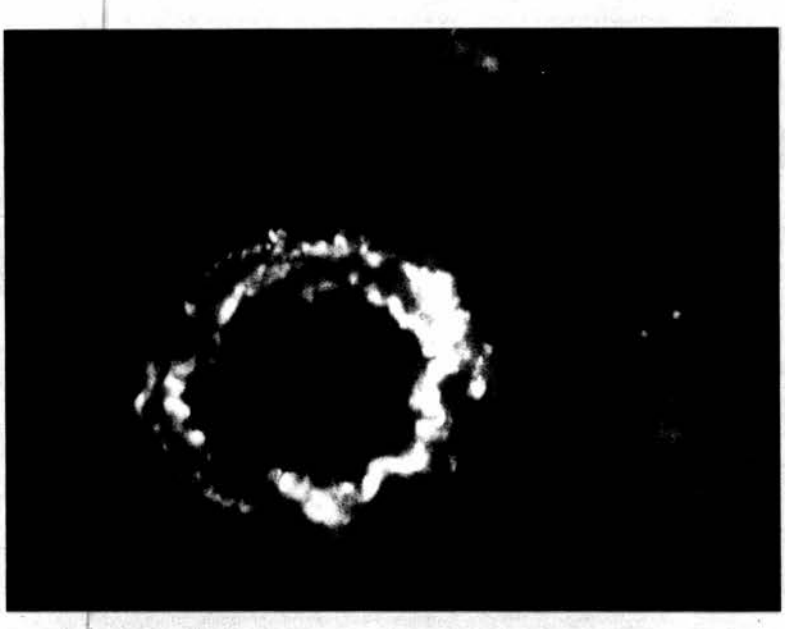
15. Scleroderma

Two patients in this study have had a diagnosis of scleroderma. The first (case 17) was a sixty-three year old female with a long history of Raynaud's phenomenon. For two years she had noticed difficulty in eating solids and she subsequently developed peripheral pitting oedema. She complained of breathlessness and a chest X-ray revealed a diffuse interstitial fibrosis. The second patient (case 235) was a sixty-six year old male who presented with hypertension and frontal headaches. Over a two month period he developed anorexia and nausea which proceeded to dyspnoea, thirst, lethargy and weakness. He was admitted to hospital in acute renal failure.

Renal biopsy on these two patients revealed changes consistent with scleroderma (II 4.n). There was a mucinous thickening of the intima of small arterioles and interlobular arteries and on immunofluorescence microscopy these were shown to contain deposits of IgM and C_3 (Fig. 81).

The first patient was treated with corticosteroids and made a satisfactory improvement. Although she continued to have difficulty with swallowing she is normotensive with good renal function and no proteinuria. The second patient, in spite of haemodialysis, made no recovery from his acute renal failure and died shortly thereafter.

Figure 81



Complement (C_3) in the thickened intima of an arteriole from a patient with scleroderma.

Case 235 Complement (C_3) x 350

16. HYPERTENSION

In this study renal biopsy was carried out in twenty-one patients with hypertension. The of these patients had malignant hypertension as evidenced by papilloedema on fundoscopic examination. There were eight females and thirteen males in this group and the mean age was thirty-five years (range 9 to 54 years).

There was a wide range of clinical presentation. In four patients the hypertension was detected during a routine examination. Two patients had a past history of renal disease; case 23 had Henoch-Schonlein purpura several years previously and case 192 had an episode of glomerulonephritis nineteen years previously. In five patients the presentation was on account of left ventricular failure. One patient complained of dizziness and one patient developed a cerebrovascular accident. Three patients complained of visual problems and two developed headaches. One patient was found to be hypertensive during pregnancy, and at post natal examination the blood pressure was still elevated. In all patients the diastolic pressure was greater than 100 mm Hg in spite of the fact that several were on hypotensive drugs prior to their initial recording being taken in hospital. In seven patients there was significant proteinuria, ranging from 1.5 to 3.9 grams. However, haematuria was present in only three patients. There was a wide range of creatinine clearance. Two patients had proven Conn's syndrome and both had a very satisfactory response to surgical excision of the adrenal adenoma. One patient (case 201) was found to have fibromuscular hyperplasia of the renal artery and had an initial good response following unilateral nephrectomy, but subsequently became

moderately hypertensive again. Three of the nine patients with malignant hypertension responded satisfactorily to hypotensive therapy; they all had good renal function at presentation. However, in seven patients there was a rapid decline in renal function, requiring treatment by intermittent haemodialysis. Four of these patients died very shortly after the initiation of this treatment.

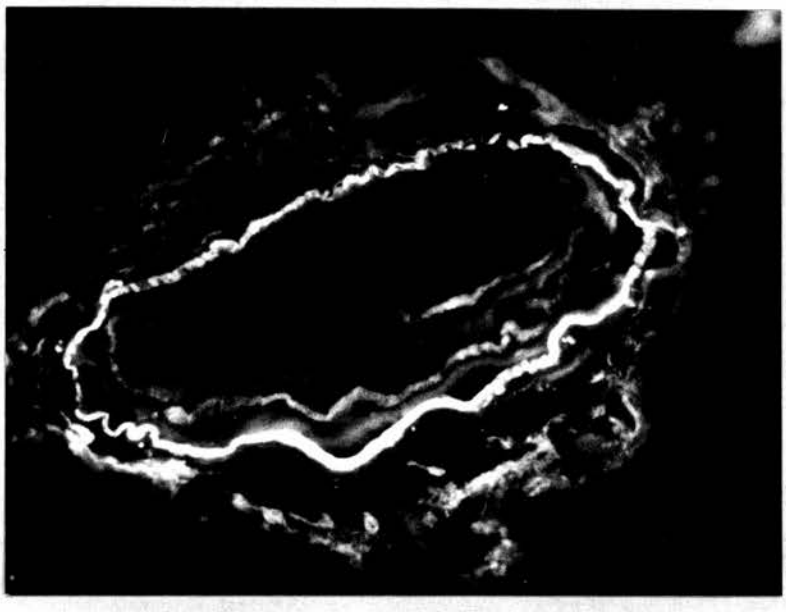
Immunofluorescence examination of renal biopsies in these patients revealed a minor deposition of fibrin/fibrinogen in a granular fashion in glomerular capillary walls in five patients. In two patients this was associated with a very small amount of IgG and in two patients with IgM; all these patients had malignant hypertension. An interesting finding was the presence of what appeared to be IgM-containing cells in the glomeruli of four patients. This was not associated with any significant polymorph infiltration on light microscopy. The most striking feature on immunofluorescence was the autofluorescence of the reduplicated and split elastic lamina in arterioles (Fig. 82). There was no difficulty in recognising the yellow or yellow-brown autofluorescence from the apple green colour of fluorescein.

On light microscopy the most significant finding was the presence of hypertensive changes in arterioles. This was associated in a significant number of patients with a mild proliferation of mesangial cells, and in some cases evidence of endothelial cell swelling. In patients with malignant hypertension there was evidence of fibrinoid necrosis in arteriolar walls.

In this study there appeared to be a considerable number of patients with hypertension as a "final" diagnosis. The prognosis in

these patients, particularly those in a malignant phase, was poor.

Figure 82



Autofluorescence of the thickened and split elastic lamina of an artery in a patient with malignant hypertension.

Case 204 Autofluorescence x 350

17. TRANSPLANT

Twenty-two biopsies have been examined from transplanted kidneys in eighteen patients. In ten instances the biopsy was obtained from eight patients because of primary non-function. Eight biopsies were obtained from six people during a rejection episode. In three instances the material was obtained at post mortem.

The biopsies obtained during investigation of primary non-function were taken on average thirty-four days after transplantation (range 21 to 52 days). On immunofluorescence microscopy the most common finding was a diffuse scattering of cells exhibiting IgM within the interstitium. In one instance these cells appeared to contain complement (C_3) in addition to IgM.

In those biopsies undertaken as part of an investigation of a possible rejection episode the most common immunofluorescent finding was that of IgM, complement (C_3) and fibrin/fibrinogen within small arterioles (Fig. 83). In addition, in three instances there were IgM containing cells in the interstitium (Fig. 84) and in three cases there was a significant amount of fibrin/fibrinogen within glomerular capillary walls. One case showed extensive deposition of IgG, IgA, IgM, C_3 and fibrin within glomerular capillary walls and to a lesser extent within the mesangial regions. This was not considered to be a re-activation of a glomerulonephritis in view of the fact that the cause of the chronic renal failure was polycystic renal disease.

In three cases tissue was obtained at post mortem. In the first (case 72) the material was obtained two days after transplantation and following death from hyperkalaemia. In this instance the only immunofluorescence finding was of a small number of IgM containing

cells within the interstitium. In the second case (case 188) renal transplantation had been performed seven years previously because of bilateral hypernephroma. Immunofluorescence examination of this specimen revealed no deposition of any immunofluorescent material throughout the specimen. In the remaining case material was obtained at post mortem some nine months after transplantation, and the only abnormality was an infiltration of a few IgM-containing cells within the interstitium.

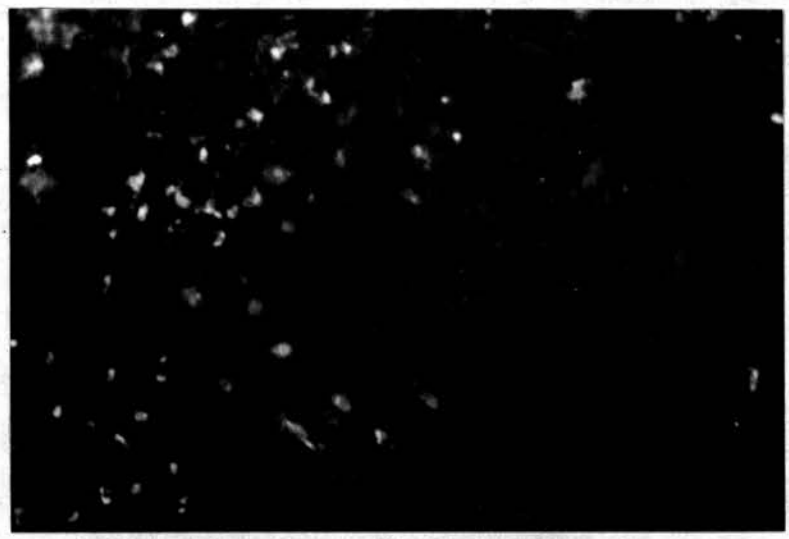
Immunofluorescence examination of material obtained from renal transplants was surprisingly unrevealing. In those patients who presented with primary non-function there was a considerable similarity to the findings obtained in acute tubular necrosis. During rejection episodes there appeared to be more material visible in small arterioles but this was by no means a consistent or universal finding. The most prominent appearance in any of the specimens was that of an infiltrate of IgM-containing cells within the interstitium; they were found very shortly after transplantation and seemed to persist for a long time thereafter. In no case was there any evidence of recurrence of the original pathology in the transplanted kidney.

Figure 83

Fibrin/fibrinogen in the wall of a longitudinally cut blood vessel and a small peritubular capillary in a transplant biopsy.

Case 185 Fibrin/fibrinogen x 350

Figure 84



Numerous IgM containing cells in the interstitium of a renal transplant biopsy. Note the lack of immunofluorescence in the glomerulus.

Case 92 IgM x 275

18. DIABETES MELLITUS

Thirteen biopsies were carried out in thirteen patients with diabetes mellitus. One patient (case 71) was pre-diabetic, two cases had maturity onset diabetes mellitus and the remainder were of the insulin dependent childhood onset type. There were eight male patients and five female patients. The average age was 34 years (range 6 to 69 years).

On initial investigation no patient exhibited haematuria. Proteinuria ranged from 2.1 grams per 24 hours to 7.9 grams per 24 hours and was present in seven patients. Hypertension was not a common feature, a diastolic pressure of 90 mm Hg or greater being present in only four patients. Renal function was variable, but in all the creatinine clearance was in excess of 30 mls per minute.

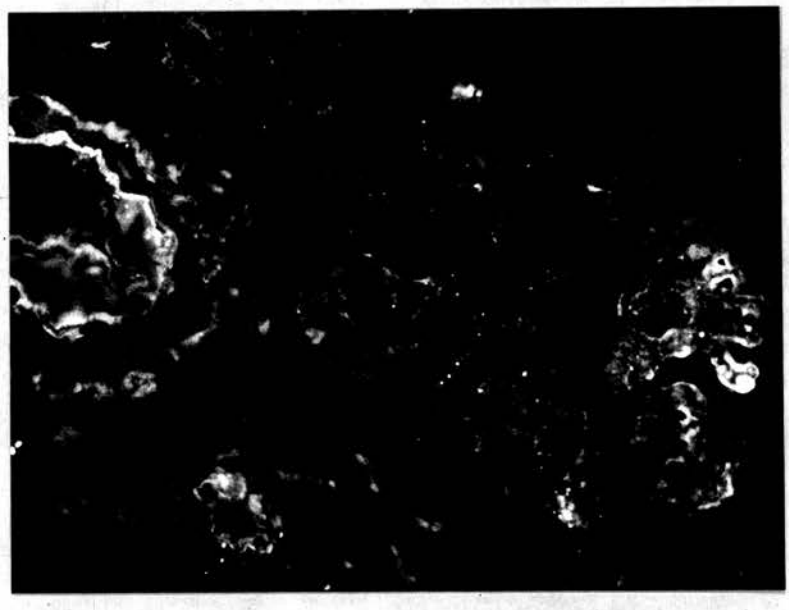
On light microscopy the most significant feature was an increase in the size of mesangial regions. In a few there was an increase in mesangial cells, but more commonly it was an increase in mesangial matrix which in some cases was large enough to produce nodule formation. In several there was evidence of capsular drops and in one there was a clear fibrin cap (Fig. 85 and 86).

On immunofluorescence microscopy small amounts of fibrin were visible in the capillary walls of six of thirteen biopsies. In one case the fibrin was present in a peripheral capillary wall and appeared to correspond to the fibrin cap seen by light microscopy (Figs. 85 and 86). This was also present in very small amounts in the mesangial regions of four biopsies. IgM was present in the capillary walls of three biopsies (Fig. 87). There was no other specific immunofluorescence in the glomerular capillary walls or

mesangial regions. In one case there was IgM present in the intima of arterioles (Fig. 88). The fibrin and IgM present in the glomerular capillary walls did not appear to bear any relationship to the time of the onset of diabetes, whether the patient was receiving insulin or oral hypoglycaemics, or whether he had proteinuria, hypertension or diminished renal function.

At follow-up examination three patients exhibited declining renal function. One died from chronic renal failure. All other patients remained relatively unchanged as far as their renal status was concerned.

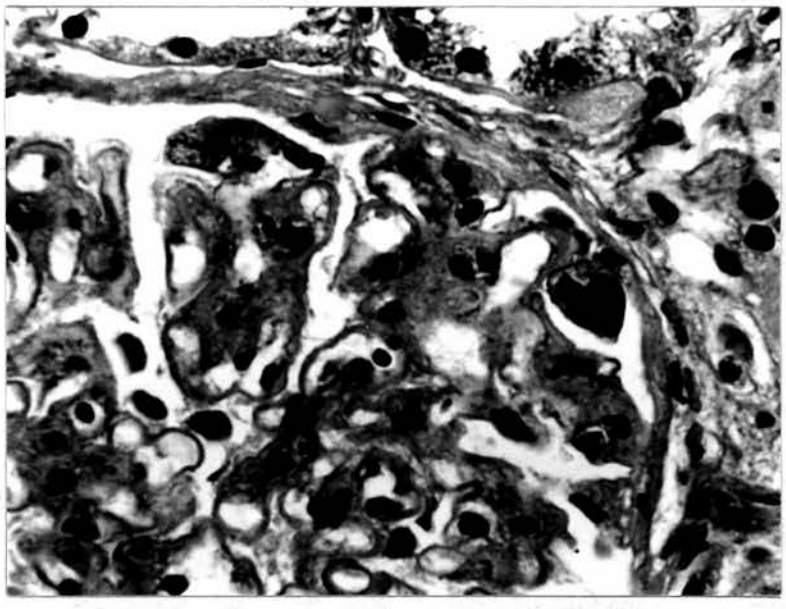
Figure 85



Small amounts of fibrin/fibrinogen in glomerular capillary walls associated with a large deposit in a peripheral glomerular capillary corresponding to a fibrin cap. The arteriole to the left exhibits autofluorescence in the elastic lamina.

Case 167 Fibrin/fibrinogen x 375

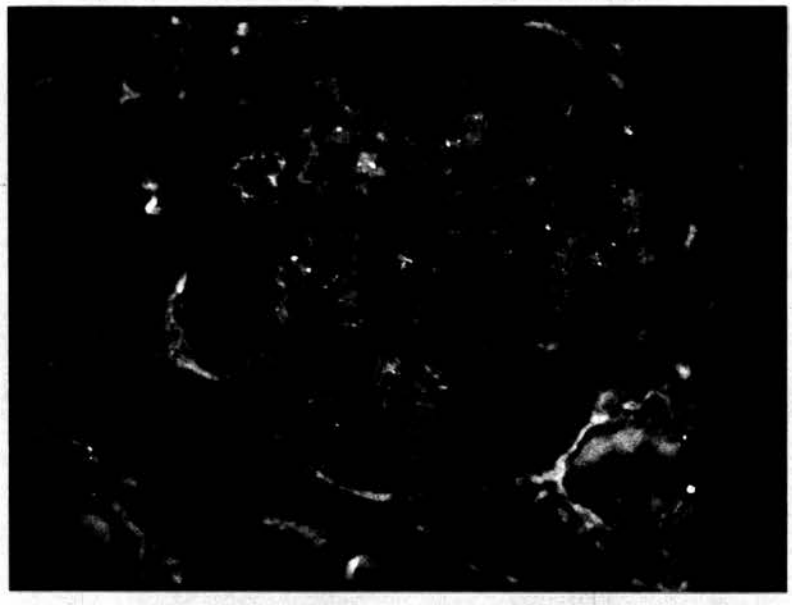
Figure 86



Diffuse diabetic glomerulosclerosis with a "fibrin cap" in a peripheral capillary.

Case 167 M.S.B. x 600

Figure 87



Diffuse diabetic glomerulosclerosis showing small amounts of IgM in glomerular capillary walls associated with a small amount of fluorescence in the mesangium.

Case 107 IgM x 375

Figure 88

Immunofluorescence to IgM in the enlarged intima of an arteriole in a patient with diffuse diabetic glomerulosclerosis.

Case 167 IgM x 450

19. AMYLOIDOSIS

Six biopsies were carried out in six patients with amyloidosis. This group consisted of four males and two females and the average age was 60.5 years (52 to 58 years). The presenting feature was nephrotic syndrome in two patients, asymptomatic proteinuria in two patients, weight loss in one patient and chronic renal failure in one patient. In three patients the amyloidosis appeared to be primary whilst in two patients it was associated with rheumatoid arthritis, and in one patient tuberculosis of the spine.

At presentation a striking feature was the lack of hypertension in spite of the high mean age. All patients had proteinuria to a variable extent but none showed haematuria. All had diminished renal function on initial investigation.

The histological appearances by light microscopy was an accumulation of homogeneous material in glomeruli, most prominently in mesangial regions, and also on the inner aspect of capillary walls. In all six patients the pale homogeneous material was also present within the wall of arterioles. This material stained positively with Congo Red dye (see Fig. 38).

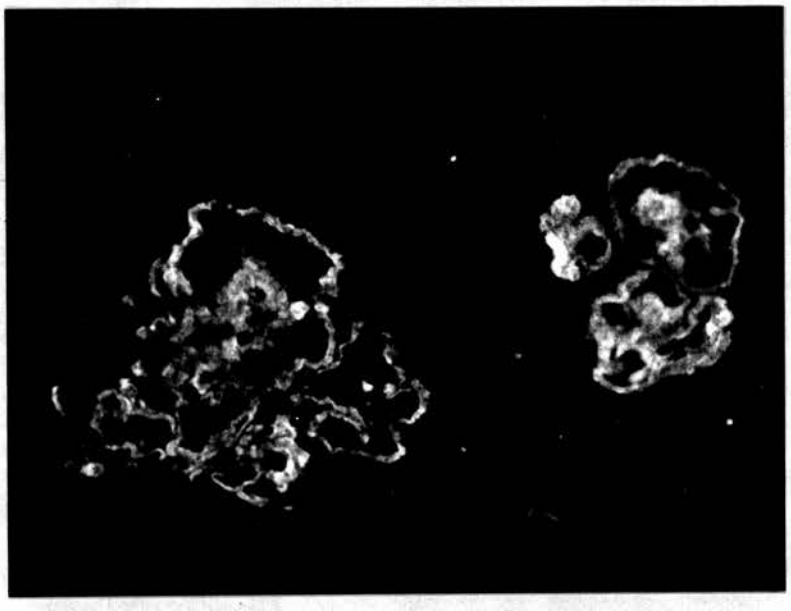
On immunofluorescence microscopy there was a surprising lack of positive findings. In one case (case 115) there was considerable deposition of IgG in expanded mesangial regions and glomerular capillary walls (Fig. 89). In addition, in this case, there was IgG present in arteriolar walls (Fig. 90). In one further case there was IgG present in small amounts in glomerular capillary walls. In three patients there was weak deposition of fibrin/fibrinogen in glomerular capillary walls. In the three cases with primary

amyloidosis no specific immunofluorescence was detected in spite of typical amyloid deposition as seen with Congo red stain.

The long term follow-up in these patients showed a very poor prognosis. Three patients died, two from cardiac failure and one from bronchopneumonia. One patient progressed to intermittent haemodialysis, while one patient remains unchanged. In the remaining patient there has been a steady deterioration in renal function.

In this series renal amyloidosis was present in six patients. In only two patients was the nephrotic syndrome present and this therefore accounts for only some 5% of adults presenting with nephrotic syndrome in this series. The prognosis in this condition is extremely poor probably due to the widespread nature of the condition.

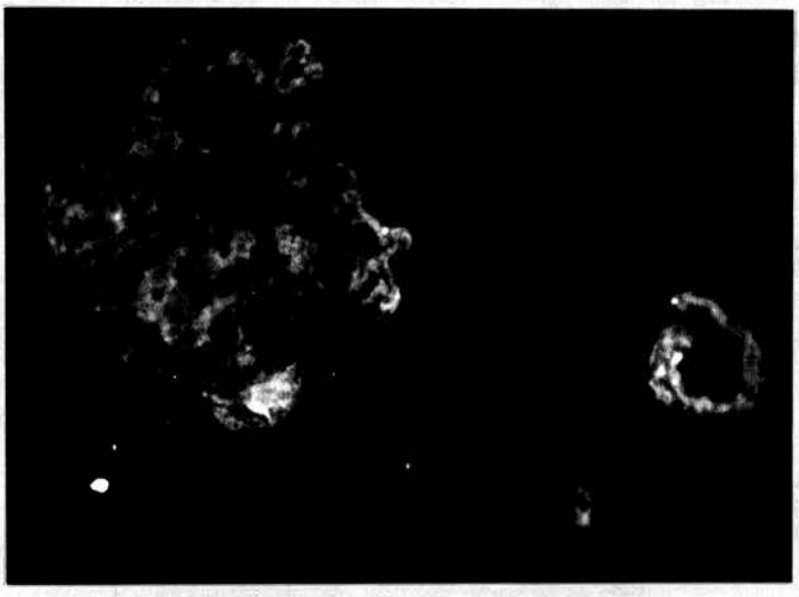
Figure 89



Immunofluorescence to IgG in expanded mesangium and glomerular capillary walls. The appearances are almost those of a membranous glomerulonephritis except that the capillary wall deposits are not so obviously granular and enlargement of the mesangium is present (compare with Figs. 13 and 58).

Case 115 IgG x 375

Figure 90



Immunofluorescence in glomerular capillary walls and also the walls of an arteriole in amyloidosis.

Case 115 IgG x 375

20. MALIGNANCY ASSOCIATED NEPHROTIC SYNDROME

In this series of three hundred biopsies one patient with a proven malignancy has been studied. This was a twenty-five year old housewife with a chorion carcinoma, and her history is described below.

In 1968 this patient had a spontaneous abortion and in the following year had an uneventful pregnancy. In January 1973 she was found to have amenorrhoea but a negative pregnancy test. Over the subsequent sixteen months she continued with amenorrhoea and lost approximately 20 kg. in weight. She was found to have increasing urinary lutinising hormone excretion and she subsequently developed massive proteinuria with a subsequent nephrotic syndrome. A renal biopsy was performed at this time; numerous capillaries in each glomerulus showed localised deeply eosinophilic hyaline thickening of the capillary walls, sometimes appearing as a large mass of hyaline or granular material continuous with the basement membrane and pushing into the capillary lumen which it almost filled (Fig. 91). Immunofluorescence examination of this showed large deposits of IgG and IgM with smaller amounts of IgA, complement (C_3) and fibrin (Figs. 92 and 93). A uterine dilatation and cauterization revealed the presence of a chorion carcinoma, and accordingly a hysterectomy was undertaken in August 1971.

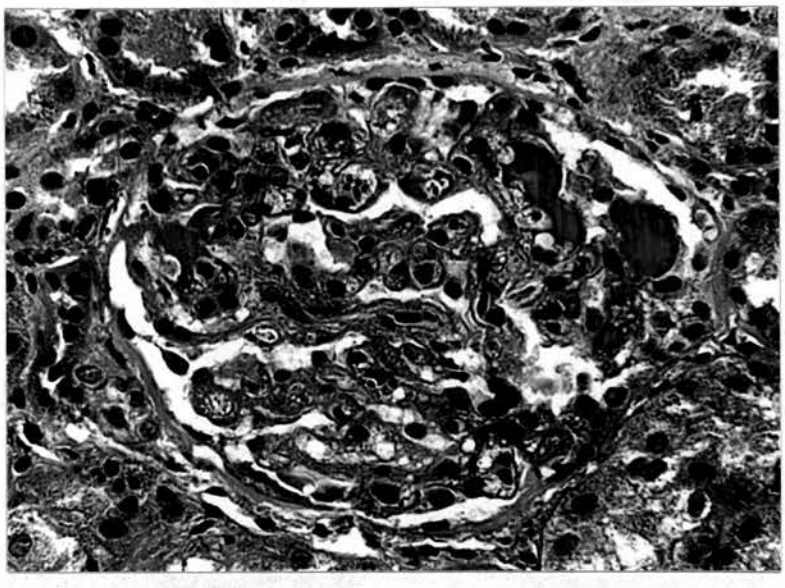
At this time she was frankly nephrotic, with a urinary protein excretion of 8.0 G per 24 hours and a creatinine clearance of 70 mls per minute. Her blood pressure was 165/100. Five months later a repeat biopsy was performed, and this showed that there was a focal increase in mesangial cells, which was never marked. The glomerular

capillary basement membrane was of normal thickness with some mesangial prominence. A few granules of material staining as for fibrin were visible on the inner aspect of the basement membrane in one or two capillaries but the large hypereosinophilic masses seen previously had entirely disappeared (Fig. 94). On immunofluorescence microscopy there was a weak deposition of IgM in the glomerular capillary walls, associated with a small amount of complement (C_3) and fibrin/fibrinogen (Fig. 95).

She continued to progress satisfactorily, and in July 1973 had normal renal function with no haematuria or proteinuria, and her blood pressure had returned to normal. A further renal biopsy at this time showed virtually normal tissue with no specific immunofluorescence.

This case illustrates a glomerulonephritis associated with a chorion carcinoma which made a satisfactory resolution following removal of the primary tumour.

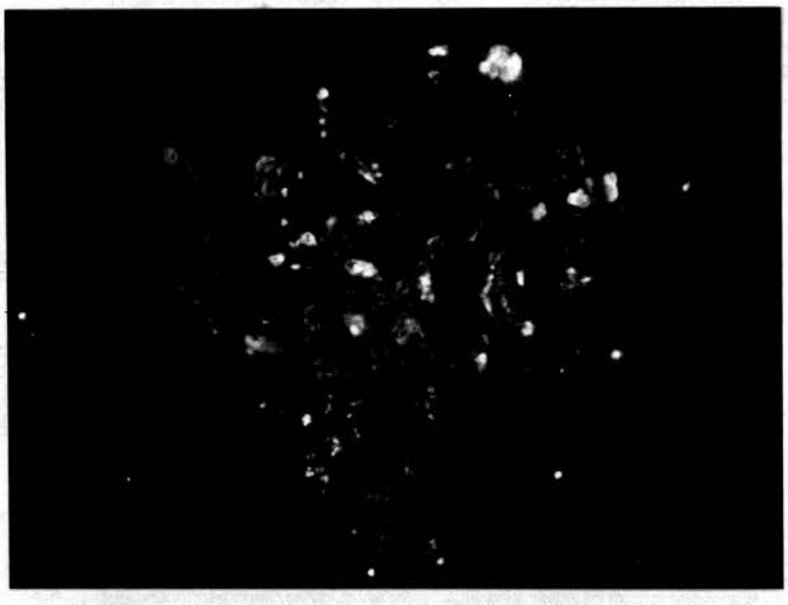
Figure 91



Localised hyaline deposits occluding the glomerular capillary lumina in a patient with chorion carcinoma and malignancy associated nephrotic syndrome.

Case 79 H and E x 400

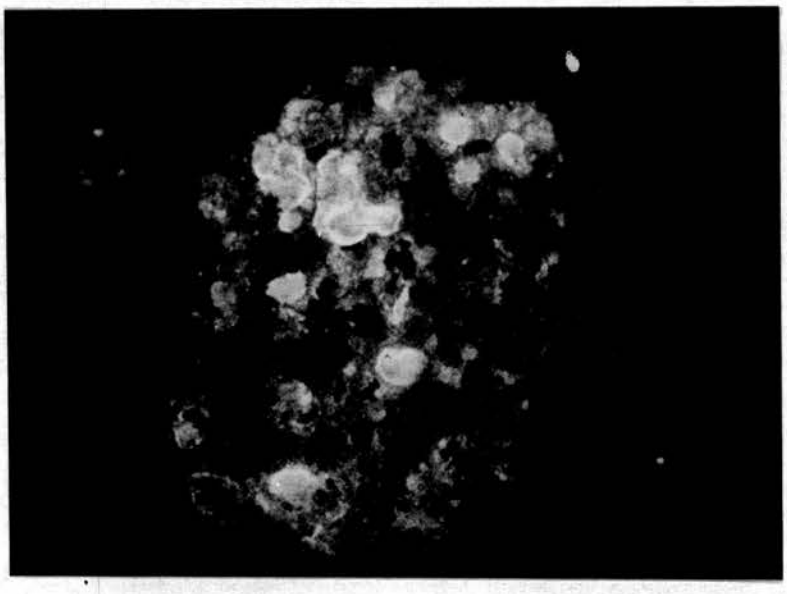
Figure 92



Immunofluorescence to IgM showing granular deposits in glomerular capillary walls with occasional large deposits almost occluding the capillary lumen.

Case 79 IgM x 375

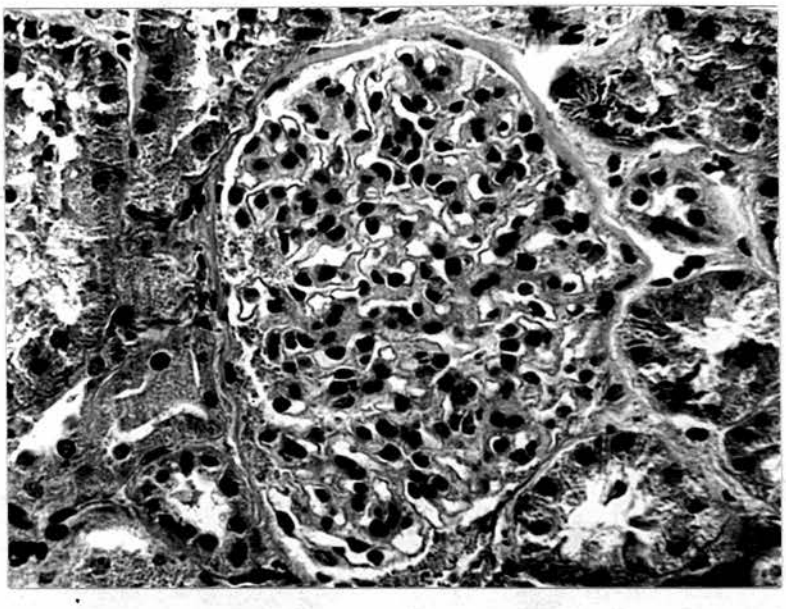
Figure 93



Large deposits of fibrin/fibrinogen which in many instances appear to occlude the glomerular capillary lumina.

Case 79 Fibrin/fibrinogen x 375

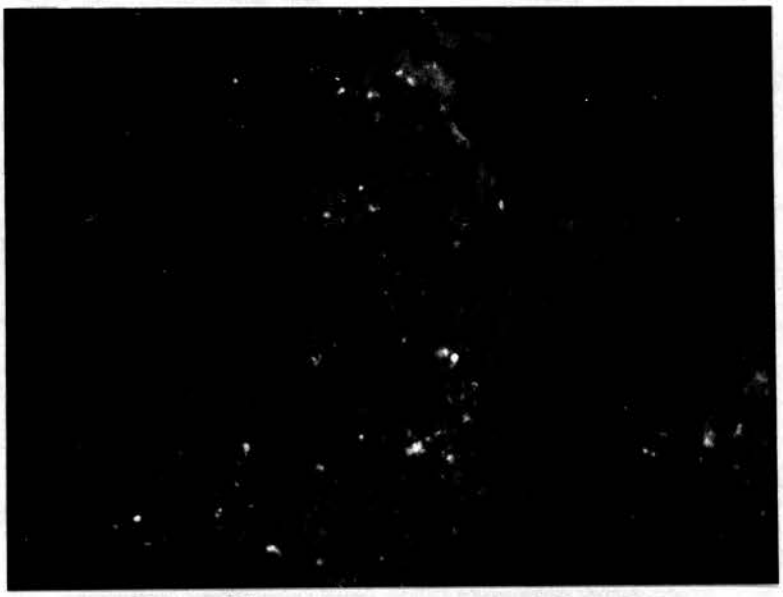
Figure 94



Repeat biopsy five months after hysterectomy in a patient with chorion carcinoma showing complete resolution of hyaline deposits and a return to almost normal histology.

Case 79 Repeat Biopsy H and E x 400

Figure 95



Repeat biopsy after hysterectomy and after resolution of renal involvement showing an almost complete clearance of the previously deposited material.

Case 79 Repeat biopsy Fibrin/fibrinogen x 600

21. MISCELLANEOUS

Fifteen biopsies have been obtained from thirteen patients with a miscellaneous spectrum of renal disease.

One case (case 223) had subacute bacterial endocarditis. This was a thirty-one year old female who presented with cough and malaise of approximately four months duration. Subacute bacterial endocarditis was diagnosed in view of changing heart murmurs, a persistently elevated ESR and positive blood cultures for Strep. viridans. On clinical examination she had predominant aortic incompetence but this was combined with a degree of aortic stenosis and a ventricular septal defect. Immunofluorescence microscopy revealed no specific immunofluorescence within the glomeruli. Light microscopy, however, showed focal glomerular hyalinisation, moderate interstitial fibrosis, and focal round cell infiltration. One arteriole showed focal mural hyalinisation with irregular fibrinoid change. This patient underwent successful aortic valve replacement with closure of her ventricular septal defect and she progresses well.

A patient with known congenital heart disease who had undergone aortic valve replacement developed non-specific arthritis, and in view of this and a mild proteinuria a renal biopsy was performed. This showed no evidence of an underlying glomerulonephritis but there was a considerable amount of haemosiderin deposited in tubular cells (Fig. 96). Immunofluorescence examination in this case revealed no specific immunofluorescence.

One patient with toxaemia of pregnancy has been studied. This was a nineteen year old female presenting in the thirty-fifth week of pregnancy with a massive concealed accidental haemorrhage.

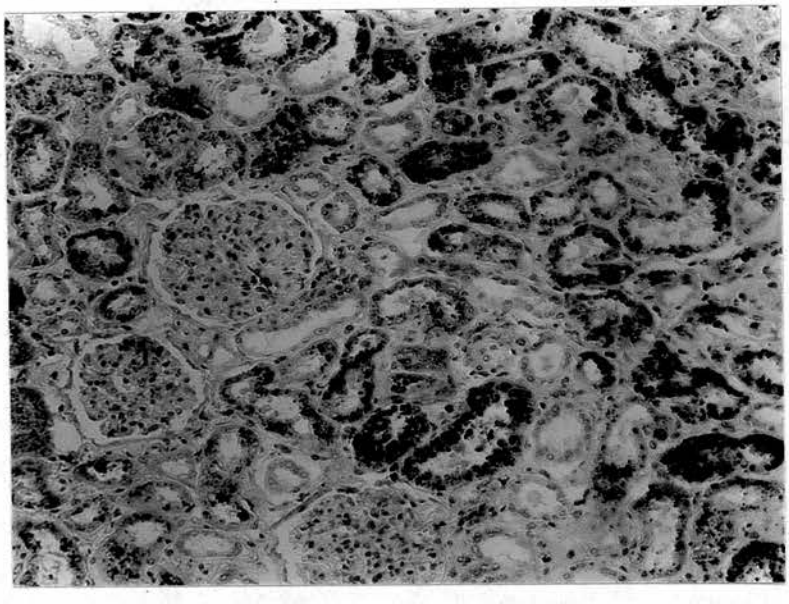
Immunofluorescence microscopy revealed widespread deposition of fibrin/fibrinogen in glomerular capillary walls. There was also deposition of IgM and a small amount of complement (C_3) in glomerular capillaries. Weak immunofluorescence to IgG was detected within the capillary walls but this was felt to be of questionable significance. Light microscopy showed no hypercellularity or polymorph infiltration, but the endothelial cells were swollen and the mesangial regions appeared prominent. Following delivery her proteinuria subsided and her renal function returned to normal.

One patient with diminished renal function due to chronic pyelonephritis underwent renal biopsy. Light microscopy showed that some six of twenty-three glomeruli were completely hyalinised and that in the remainder there was marked periglomerular fibrosis. The tubules were surrounded by a heavy chronic inflammatory infiltrate and the appearances were those of chronic pyelonephritis. On immunofluorescence microscopy, in twenty glomeruli there was a granular deposition of IgG and complement within the capillary walls and also in a few mesangial regions. The significance of these findings is unknown. At follow-up examination two years later she continues to have impaired renal function but there has been no obvious deterioration.

Renal biopsies have been performed in five patients with predominantly tubular disease. These are diabetes insipidus, Fanconi's syndrome, inappropriate ADH secretion, orthostatic proteinuria and familial juvenile nephronophthiasis. In none of these biopsies has any immunofluorescence been detected. In addition, material has been obtained at surgery from three patients.

As far as can be determined these patients had normal renal function and no proteinuria. Immunofluorescence examination of this material was negative.

Figure 96



Dark granules of haemosiderin in tubular cells, probably proximal tubules.

Case 171 P.B.R. x 160

22. IMMUNOGLOBULIN G DEPOSITION

The deposition of IgG is shown in Table 13. In ninety of the biopsies studies, IgG was present within the glomerular capillary walls, the capillary walls and mesangium, or mesangium alone.

IgG deposition was most frequent in proliferative glomerulonephritis, membranous glomerulonephritis, mesangiocapillary glomerulonephritis, focal proliferative glomerulonephritis and disseminated lupus erythematosus. It was most commonly found in the glomerular capillary walls or in the walls and mesangial regions, and seldom solely in the mesangial regions, except in the condition of mesangial IgG/IgA deposition. In all cases the deposition was granular although in some cases the small granular deposits appeared confluent and gave the appearance of short linear deposits.

In many instances the pattern of deposition was characteristic of a specific condition such as the uniform granular deposition on all glomerular capillary walls in membranous glomerulonephritis (Figs. 13 and 58) or the granular deposition in the peripheral walls of glomerular capillaries in mesangiocapillary glomerulonephritis (Fig. 59). The typical appearances are described in the relevant sections (IV 1 - 20).

TABLE 13

DISTRIBUTION OF GLOMERULAR IMMUNOFLUORESCENCE TO IgG

	Immunofluorescence deposition			No. of cases studied	
	Capillary Walls	Walls + Mesangium	Mesangium		
Diffuse Proliferative					
A. Mild	2	2	1	15	
B. Moderate	5	7 (9)	1	41	(46)
C. Exudative	4	4 (5)	1	16	(18)
D. Progressive	1	1	1	6	
Rapidly prog.	4 (5)	0	0	5	(8)
Membranous	9 (10)	1	0	16	(17)
Mesangiocapillary	5	1	0	13	(14)
Focal Prolif.	4	2 (3)	1	11	(12)
Mesangial IgG/IgA	0	0	2	2	
Minimal lesion	0	0	0	20	(24)
Henoch-Schonlein	2 (3)	2	1	11	(12)
A.T.N.	0	0	0	9	(11)
D.I.C.	1 (2)	0	0	7	(9)
D.L.E.	4	2	0	10	
Polyarteritis	3 (4)	1	0	12	(13)
Scleroderma	0	0	0	2	
Hypertension	1	1	0	21	
Transplant	1	1	0	18	(22)
Diabetes Mellitus	1	1	0	13	
Amyloidosis	1	0	1	6	

Figures are for total number excluding repeat biopsies.

Figures in brackets are for total number including repeat biopsies.

23. IMMUNOGLOBULIN A DEPOSITION

IgA was detected within fifty-nine of the three hundred biopsies studied. The distribution of this immunoglobulin is shown in Table 14.

In proliferative glomerulonephritis IgA is most commonly deposited in the mesangium alone, or in the mesangium associated with some slight deposition in the capillary walls. It is relatively infrequently found in the capillary walls alone. In three cases IgA was detected in the capillary walls of patients with membranous glomerulonephritis. In only four of the patients with Henoch-Schonlein purpura was any IgA detected in glomeruli.

In all cases the deposition was granular. In many cases casts were visible in tubules and these gave bright homogeneous immunofluorescence to IgA.

TABLE 14

DISTRIBUTION OF GLOMERULAR IMMUNOFLUORESCENCE TO IgA

	Immunofluorescence deposition			No. of cases studied
	Capillary Walls	Walls + Mesangium	Mesangium	
Proliferative				
A. Mild	2	0	2	15
B. Moderate	0	5	2 (3)	41 (46)
C. Exudative	3	3	1 (2)	16 (18)
D. Progressive	0	2	0	6
Rapidly prog.	1	0	0	5 (8)
Membranous	3	0	0	16 (17)
Mesangiocapillary	2	1	0	13 (14)
Focal Prolif.	2	1	2	11 (12)
Mesangial IgG/IgA	0	0	2	2
Minimal lesion	0	0	0	20 (24)
Henoch-Schonlein	4	1	1	11 (12)
A.T.N.	0	0	0	9 (11)
D.I.C.	1 (3)	1	0	7 (9)
D.L.E.	0	1	1	10
Polyarteritis	1	1	0	12 (13)
Scleroderma	0	0	0	2
Hypertension	1	1	0	21
Transplant	0	0	0	18 (22)
Diabetes Mellitus	0	0	0	13
Amyloidosis	1	1	0	6

Figures are for total number excluding repeat biopsies.

Figures in brackets are for total number including repeat biopsies.

24. IMMUNOGLOBULIN M DEPOSITION

IgM was detected in ninety-one of the three hundred biopsies studied. The glomerular distribution is shown in Table 15. In all cases the deposition was of a granular distribution.

IgM was most commonly noted in glomerular capillary walls, although it was also frequently found within capillary walls and mesangial regions. It was most common in cases of proliferative glomerulonephritis but was also found in membranous glomerulonephritis, mesangiocapillary glomerulonephritis and focal proliferative glomerulonephritis. Six of the transplant patients studied had IgM deposition in either the capillary walls or the capillary walls and mesangial regions.

TABLE 15

DISTRIBUTION OF GLOMERULAR IMMUNOFLUORESCENCE TO IgM

	Immunofluorescence deposition			No. of cases studied	
	Capillary Walls	Walls + Mesangium	Mesangium		
Proliferative					
A. Mild	2	2	1	15	
B. Moderate	6	8 (9)	2	41	(46)
C. Exudative	1	4 (5)	1	16	(18)
D. Progressive	2	0	3	6	
Rapidly prog.	1 (3)	0	0	5	(8)
Membranous	2 (3)	1	0	16	(17)
Mesangiocapillary	4	1	0	13	(14)
Focal Prolif.	5	3	0	11	(12)
Mesangial IgG/IgA	0	0	0	2	
Minimal lesion	2 (3)	1	0	20	(24)
Henoch-Schonlein	1	1	0	11	(12)
A.T.N.	2	0	0	9	(11)
D.I.C.	2 (3)	1	0	7	(9)
D.L.E.	3	0	1	10	
Polyarteritis	1	1	1	12	(13)
Scleroderma	0	0	0	2	
Hypertension	1	1	1	21	
Transplant	4	2	0	18	(22)
Diabetes Mellitus	1	2	0	13	
Amyloidosis	2	1	0	6	

Figures are for total number excluding repeat biopsies.

Figures in brackets are for total number including repeat biopsies.

25. IMMUNOGLOBULIN E DEPOSITION

Fluorescene labelled anti IgE serum was available to study forty of the biopsies in this series. In only one case (case 204, Diffuse Proliferative Glomerulonephritis) was there any IgE deposition detected. In this biopsy only small amounts were present in the mesangial regions in one of seven glomeruli. The cases studied are shown in Table 16.

TABLE 16

PATIENTS STUDIED WITH IgE ANTISERUM

Proliferative Glomerulonephritis	
resolving	4
mild	6
acute	2
progressive	3
Membranous Glomerulonephritis	1
Mesangiocapillary Glomerulonephritis	1
Minimal lesion	3
Henoch-Schonlein	1
D.L.E.	1
Hypertension	7
Transplant	9
SBE	1
Normal	1

26. IMMUNOGLOBULIN D DEPOSITION

Anti serum to IgD was available for the study of seventy-six biopsies towards the end of this present series. No specific immunofluorescence to IgD was detected in any of the biopsies examined. The range of cases studied is shown in Table 17.

TABLE 17

PATIENTS STUDIED WITH IgD ANTISERUM

Proliferative Glomerulonephritis	
mild	18
acute	6
rapidly progressive	3
Membranous Glomerulonephritis	9
Mesangiocapillary Glomerulonephritis	4
Focal Proliferative Glomerulonephritis	4
Mesangial IgG/IgA	1
Minimal Lesion	6
Focal Glomerulosclerosis	1
Henoch-Schonlein	3
Acute Tubular Necrosis	3
D.L.E.	4
Polyarteritis	3
Miscellaneous	11

27. COMPLEMENT (C_3) DEPOSITION

Complement (C_3) was detected in seventy-five of the three hundred biopsies examined. This distribution is shown in Table 18.

Complement (C_3) was most frequently detected in glomerular capillary walls. In only eleven cases was it detected solely in the mesangial regions. It was commonly found in proliferative glomerulonephritis, but was also a frequent finding in cases of membranous and mesangiocapillary glomerulonephritis.

TABLE 18

DISTRIBUTION OF GLOMERULAR IMMUNOFLUORESCENCE TO COMPLEMENT (C_3)

	Immunofluorescence deposition			No. of cases studied	
	Capillary Walls	Walls + Mesangium	Mesangium		
Proliferative					
A. Mild	2	1	1	15	
B. Moderate	7	4 (5)	3	41	(46)
C. Exudative	2	5	2	16	(18)
D. Progressive	1	2	2	6	
Rapidly prog.	2	0	0	5	(8)
Membranous	4 (5)	0	0	16	(17)
Mesangiocapillary	4	2	0	13	(14)
Focal Prolif.	2	2	0	11	(12)
Mesangial IgG/IgA	0	0	1	2	
Minimal lesion	1	0	0	20	(24)
Henoch-Schonlein	2	1	0	11	(12)
A.T.N.	2	0	0	9	(11)
D.I.C.	1	1	0	7	(9)
D.L.E.	1	1	0	10	
Polyarteritis	1 (2)	2	0	11	(12)
Scleroderma	0	0	0	2	
Hypertension	1	0	0	21	
Transplant	1	1	1	18	(22)
Diabetes Mellitus	0	0	0	13	
Amyloidosis	1	0	0	6	

Figures are for total number excluding repeat biopsies.

Figures in brackets are for total number including repeat biopsies.

28. COMPLEMENT (C_4) DEPOSITION

Towards the end of the present study antiserum to the fourth component of complement was available. This was used for studying seventy-seven cases, and C_4 deposition was detected in eleven cases. It was most commonly present in membranous glomerulonephritis, where it was present in four out of nine cases studied. In only three of the eighteen patients with mild to moderate proliferative glomerulonephritis was C_4 detected. It was also present within two of four cases of mesangiocapillary glomerulonephritis and one of four cases of focal proliferative glomerulonephritis. In nine instances the C_4 deposition was confined to the glomerular capillary walls. In one it was present in the capillary walls and in the mesangial regions, and in one it was in the mesangium alone.

TABLE 19

DISTRIBUTION OF GLOMERULAR IMMUNOFLUORESCENCE TO COMPLEMENT (C_4)

	Immunofluorescence deposition			No. of cases studied
	Capillary Walls	Walls + Mesangium	Mesangium	
Proliferative				
A. Mild	0	0	0	0
B. Moderate	2	1	0	18
C. Exudative	0	0	1	6
D. Progressive	0	0	0	0
Rapidly prog.	0	0	0	3
Membranous	4	0	0	9
Mesangiocapillary	2	0	0	4
Focal Prolif.	1	0	0	4
Mesangial IgG/IgA	0	0	0	1
Minimal lesion	0	0	0	6
Henoch-Schonlein	0	0	0	3
A.T.N.	0	0	0	3
D.I.C.	0	0	0	1
D.L.E.	0	0	0	4
Polyarteritis	0	0	0	3
Scleroderma	0	0	0	1
Hypertension	0	0	0	4
Transplant	0	0	0	2
Diabetes Mellitus	0	0	0	0
Amyloidosis	0	0	0	0

29. FIBRIN/FIBRINOGEN DEPOSITION

Fibrin/fibrinogen was the material detected most commonly in this study. It was found in one hundred and twenty-six of three hundred biopsies. Only eight of these biopsies were repeat biopsies, so even with the exclusion of these this is still a very high incidence. It was present most often in glomerular capillary walls, and in only four instances was it found solely within mesangial regions (Table 20).

Fibrin/fibrinogen appeared to have a wide distribution in the cases studied, but was most common in proliferative glomerulonephritis. However, it was frequently noted in transplant biopsies, in all cases of disseminated intravascular coagulation and in a considerable number of patients with minimal lesion glomerulonephritis.

Fibrin/fibrinogen deposition would therefore appear to be common in a wide variety of glomerular diseases. An opportunity was taken during this study to assess the most accurate method of detecting intrarenal fibrin deposition. A comparison was made between the detection of fibrin/fibrinogen by immunofluorescence and the assessment of fibrin deposition as observed in sections stained by routine histological fibrin stains and by electron microscopy. These results were correlated with the urinary fibrin/fibrinogen degradation product (F.D.P. excretion).

Histological preparations were stained by picro Mallory V (P.M.) method employing acid fuchsin (acid violet 19) and by the Martius Scarlet Blue (M.S.B.) method, employing brilliant crystal scarlet 6R (acid red 44) of Lendrum et al. 1962. The distribution of red staining was recorded on the following scale:- O no positive material, + positive material in fewer than half of glomeruli,

++ positive material in more than half of the glomeruli and +++ large deposits of positive material in glomeruli and also frequently in blood vessels. Table 21 shows the histological grading as estimated by fibrin stains compared with the immunofluorescence microscopy grading employing specific FITC anti-human fibrin/fibrinogen serum. MSB positive material was present in only twenty-four of sixty-two sections in which fibrin was detected by immunofluorescence and in nine of thirty-six sections in which there was no specific immunofluorescence. Examples of positive immunofluorescence with negative MSB staining was found in all histological groups. Negative immunofluorescence and positive MSB staining occurred in glomerulonephritis, amyloidosis and diabetic glomerulosclerosis.

Electron microscopic assessment of fibrin deposition was assessed on the following scale:- 0 no abnormal deposits, + a rarefied layer between the basement membrane and the endothelium containing strands of osmiophilic material, ++ the presence in addition of definite focal deposits of dark fibriller material between the basement membrane and the endothelium, +++ similar but more abundant material sometimes present also in the capillary lumen and often associated with mesangial reaction, ++++ complete occlusion of a glomerular capillary lumen by thrombus. Table 22 shows the electron microscopic grading compared with the MSB and picro Mallory staining. The results obtained were essentially similar to those obtained with immunofluorescence except that there were fewer cases of positive histological staining in the absence of electron microscopic detection of fibrin. Table 23 shows the electron microscopic assessment compared with the maximum urinary FDP excretion.

The correlation between these two measurements is good ($p < 0.001$) but with a slight tendency towards over assessment by electron microscopy.

The degree of fibrin deposition was assessed by immunofluorescence according to the following scale:- 0 no immunofluorescence, + positive material present in the glomerular capillary wall alone or mesangium alone, ++ material present in glomerular capillary wall and mesangium, +++ glomerular capillary occlusion, ++++ deposits within the glomeruli and elsewhere such as epithelial crescents, interstitial spaces or peritubular blood vessels. Table 24 shows the relationship between the electron microscopy and the immunofluorescence findings. There is a wide distribution, again with evidence of over assessment by electron microscopy. Twelve patients graded as ++ or greater by electron microscopy had no fibrin detectable by immunofluorescence. Table 25 shows the immunofluorescence grading of intrarenal fibrin deposition and the maximum urinary FDP concentration. The relationship between these two methods is highly significant ($p < 0.001$).

This shows the limitations of the routine histological stains for the detection of intra glomerular fibrin deposition. In addition it highlights the fact that there may be an over diagnosis of fibrin when the criteria described in electron microscopic examination are used alone. Assuming urinary fibrin/fibrinogen degradation product excretion to be the most accurate method of detecting intraglomerular fibrin deposition it would appear as though immunofluorescence, employing specific anti-fibrin/fibrinogen serum, is the most accurate method for detecting the glomerular distribution.

TABLE 20

DISTRIBUTION OF GLOMERULAR IMMUNOFLUORESCENCE TO FIBRIN/FIBRINOGEN

	Immunofluorescence deposition			No. of cases studied	
	Capillary Walls	Walls + Mesangium	Mesangium		
Proliferative					
A. Mild	5	1	0	15	
B. Moderate	15	5	0	41	(46)
C. Exudative	5 (6)	4 (5)	3	16	(18)
D. Progressive	1	4	0	6	
Rapidly prog.	4 (5)	0	0	5	(8)
Membranous	8	1	0	16	(7)
Mesangiocapillary	4	2	0	13	(14)
Focal Prolif.	2	2	0	11	(12)
Mesangial IgG/IgA	2	0	0	2	
Minimal lesion	4 (6)	2	0	20	(24)
Henoch-Schonlein	4	1	0	11	(12)
A.T.N.	4	0	0	9	(11)
D.I.C.	4 (6)	1	0	7	(9)
D.L.E.	2	5	0	10	
Polyarteritis	5 (6)	2	0	12	(13)
Scleroderma	0	0	0	2	
Hypertension	4	1	1	21	
Transplant	6	1	0	18	(22)
Diabetes Mellitus	2	4	0	13	
Amyloidosis	2	1	0	6	

Figures are for total number excluding repeat biopsies.

Figures in brackets are for total number including repeat biopsies.

TABLE 21

DETECTION OF FIBRIN BY IMMUNOFLUORESCENCE MICROSCOPY AND HISTOLOGICAL STAINING

Immuno-fluorescence	Martius-Scarlet Blue				Picro-Mallory			
	0	+	++	+++	0	+	++	+++
0	27	6	3		19	3	3	
+	20	6	3		15	3	4	
++	9	5	1		4	6	1	
+++	5	2		1	2	3	1	1
++++	4	4	2		5	2	2	

TABLE 22

ELECTRON MICROSCOPIC GRADING OF FIBRIN AND HISTOLOGICAL STAINING

E.M. grading	Martius-Scarlet Blue				Picro-Mallory			
	0	+	++	+++	0	+	++	+++
0	6		2		4	1	1	
+	14	5			12		3	
++	17	2			12	2	2	
+++	13	7	2		8	10	2	
++++	4	5	1		2	2	2	

TABLE 23

RELATION BETWEEN URINARY FIBRIN/FIBRINOGEN DEGRADATION PRODUCTS AND EXTENT OF INTRAGLOMERULAR FIBRIN DEPOSITION AS JUDGED BY ELECTRON MICROSCOPY

Glomerular Fibrin Electron Microscopic Grading	Maximum Urine FDP Concentration (ug/ml)					
	0-1	1-2	2-5	5-10	10-20	>20
0	6	2		1		
+	5	4	6	1		1
++	5	3	2	2	1	2
+++	3	2	1	4	3	4
++++	1	1	6		1	2

TABLE 24

DETECTION OF FIBRIN BY IMMUNOFLUORESCENCE MICROSCOPY

AND ELECTRON MICROSCOPY

Immuno-fluorescence	Electron Microscopic Grading				
	0	+	++	+++	++++
0	8	7	6	5	1
+	1	8	8	5	4
++	2	2	3	4	5
+++		1	1	5	1
++++		1	2	5	1

TABLE 25

RELATION BETWEEN URINARY FIBRIN/FIBRINOGEN DEGRADATION

PRODUCTS AND IMMUNOFLUORESCENCE TO FIBRIN/FIBRINOGEN

Fibrin/Fibrinogen Immuno-fluorescence	Maximum Urine FDP Concentration (ug/ml)					
	0-1	1-2	2-5	5-10	10-20	>20
0	20	8	1	1		
+	9	6	7	1	2	
++	2		9	3		
+++			1	3	3	2
++++				2	3	6

30. CLINICAL PRESENTATION AND FINAL DIAGNOSIS

The clinical presentation and the final diagnosis in 208 patients are shown in Table 26. Excluded from this table are patients with known conditions such as diabetes mellitus; transplantation: repeat biopsy studies; and patients treated with steroids or Indomethacin at the time or just prior to biopsy. The clinical presentation used is that at onset of symptoms rather than at the time of biopsy. This is in view of the fact that many people had been treated adequately with hypotensives and diuretic agents and so were not nephrotic or hypertensive at the time of biopsy.

The interesting feature derived from this table is the wide underlying diagnosis that may present in a single manner. For instance, patients with nephrotic syndrome may have any of eleven different underlying diagnoses. This list, of course,is not complete, in as much as several conditions such as the connective tissue diseases and diabetes mellitus have been excluded from this table. Nevertheless it emphasises the importance of taking all factors,including renal biopsy, into account when making the final diagnosis.

TABLE 26

CLINICAL PRESENTATION AND FINAL DIAGNOSIS

208 PATIENTS

	Asymptomatic Proteiruria	Nephrotic Syndrome	Acute Nephritis	Recurrent Haematuria	Asymptomatic Haematuria	Acute Renal Failure	Chronic Renal Failure	Hypertension	? Systemic Disease	Henoch-Schonlein
Diffuse Proliferative	15	18	14	13	2			3	1	
Rapidly Progressive						6				
Membranous	2	14								
Mesangiocapillary	2	6	3					2		
Focal Proliferative	2	4	2		1			1	1	
Mesangial IgG/IgA	1			1						
Minimal		20								
Focal Glomerulosclerosis	1	2						1	1	
Henoch-Schonlein			1	1						9
Acute Tubular Necrosis						9				
D.I.C.						4				
Goodpastures Syndrome						1				
S.L.E.		1							4	
Polyarteritis						1			9	
Scleroderma						1			1	
Hypertension						1	2	17		
Amyloidosis	2	2					1			
Orthostatic Prot.	1									
Pyelonephritis							1			

31. CLINICAL PRESENTATION AND HISTOLOGICAL DIAGNOSIS

The clinical presentation and the underlying histological diagnosis obtained at biopsy is shown in Table 27. The histological diagnosis obtained at first biopsy in the 208 patients is taken for the purposes of this table.

This table again reveals the widespread clinical presentation of single histological appearances. For instance progressive proliferative glomerulonephritis may be the underlying histological diagnosis in eight different clinical presentations. The only consistent finding appears to be that acute tubular necrosis presents only as acute renal failure!

TABLE 27

CLINICAL PRESENTATION AND HISTOLOGICAL DIAGNOSIS

208 PATIENTS

	Asymptomatic Proteinuria	Nephrotic Syndrome	Acute Nephritis	Recurrent Haematuria	Asymptomatic Haematuria	Acute Renal Failure	Chronic Renal Failure	Hypertension	? Systemic Disease	Henoch-Schonlein
Proliferative mild	14	23	6	12	2			2		3
moderate		3	1	1						
exudative			6						1	1
progressive	2	3	2	2		1		3	3	2
Rapidly progressive						6			1	1
Membranous	2	14								
Mesangiocapillary	2	6	3					2		
Focal Proliferative	2	4	2		1			1	1	2
Minimal		10								
Focal Glomerulosclerosis	1	1								
Acute Tubular Necrosis						9				
D.I.C.						4				
S.L.E.		1							3	
Polyarteritis						1			7	
Scleroderma						1			1	
Hypertension						1	2	15		
Amyloidosis	2	2					1			
Pyelonephritis							1	1		
Normal	1									

32. HISTOLOGICAL DIAGNOSIS AND FINAL DIAGNOSIS

The histological diagnosis and final diagnosis in 177 cases is shown in Table 28.

Although this table has a better correlation it is by no means absolute. This only reflects the fact that in reaching the final diagnosis in many patients with renal disease the renal biopsy findings are crucial. However, it is interesting to note that even with a histological diagnosis of mild proliferative glomerulonephritis the final diagnosis may be diffuse proliferative glomerulonephritis, mesangial IgG/IgA disease, minimal lesion glomerulonephritis or Henoch-Schonlein syndrome. This again emphasises the point that in reaching a final diagnosis all facts relating to the patient must be considered.

TABLE 28

HISTOLOGICAL DIAGNOSIS AND FINAL DIAGNOSIS

177 PATIENTS

	Diffuse Proliferative	Rapidly Progressive	Membranous	Mesangiocapillary	Focal Proliferative	Mesangial IgG/IgA	Minimal	Focal Glomerulosclerosis	Henoch-Schonlein	Acute Tubular Necrosis	D.I.C.	S.L.E.	Polyarteritis
Proliferative mild	43					2	10		5				
moderate	5												
exudative	7								1				
progressive	10							3	2			1	1
Rapidly progressive		6							1				1
Membranous			16										
Mesangiocapillary				13									
Focal Proliferative					11				2				
Minimal							10						
Focal Glomerulosclerosis								2					
Acute Tubular Necrosis										9			
D.I.C.											4		
S.L.E.												4	
Polyarteritis													8

Excluding a final diagnosis of Hypertension, Chronic Pyelonephritis, Amyloidosis, Goodpasture's Syndrome, Scleroderma, Orthostatic proteinuria.

33. REPEAT STUDIES

In this study repeat studies were carried out in twenty-five patients. In twenty-one instances two biopsies were performed and in four instances three biopsies were performed.

The repeat biopsies were performed on average thirty-four weeks after the initial biopsy (range 1 to 90 weeks). In three instances the repeat biopsy was performed because the initial specimen yielded insufficient tissue for accurate diagnosis by either light, electron or immunofluorescence microscopy. In four instances the repeat specimen was obtained from a nephrectomy specimen, and in two further instances the material came from a post mortem examination.

The indications for repeat biopsy were 1) to determine the effect of therapy (6 cases), 2) to study the progression of a disease process (5 cases), 3) following an improvement in function after acute renal failure (4 cases), 4) to study a transplant rejection (4 cases), 5) because insufficient material was obtained on the initial biopsy (3 cases), 6) remission of the nephrotic syndrome (2 cases), and 7) because a nephrectomy was performed for post biopsy bleeding (1 case).

In five instances the repeat biopsy was performed following a course of Indomethacin therapy. In four of the patients the initial diagnosis was a minor proliferative glomerulonephritis and following the Indomethacin therapy the glomerular features were relatively unchanged apart from the fact that in two patients the proliferation appeared less although there was a slight increase in mesangial matrix. One patient had a moderate proliferative glomerulonephritis following an acute nephritic illness. After treatment with Indomethacin the histological appearances were improved in as much as there was less

proliferation but again the mesangial matrix appeared more prominent. On immunofluorescence microscopy in these patients there was less immunofluorescence in the second post treatment biopsy than in the initial specimen. In no instances did the second biopsy contain either immunoglobulins, complement or fibrin if this had not been present in the initial biopsy. For example Case 132 had IgG, IgM, complement (C_3) and fibrin in glomerular capillary walls on initial biopsy and after some thirty-six weeks treatment with Indomethacin there was only slight mesangial IgG deposition. In none of these five patients did there appear to be any change in their underlying renal function. The remaining patient who was studied after therapy was case 79 who had a malignancy associated nephrotic syndrome and who had two biopsies at twenty-four and seventy-seven weeks after hysterectomy. This case is fully described in Section 19.

In four cases the repeat renal biopsy was performed after an improvement in renal function following a period of acute renal failure. In the first (case 10) acute tubular necrosis was followed by an adequate return of renal function. Unfortunately following this the patient had a cardiac arrest and died. Initially there was evidence of acute tubular necrosis and by immunofluorescence there was an infiltration of cells containing IgG, IgA and IgM in the interstitium. The post mortem tissue showed some improvement in the histological appearances of acute tubular necrosis but there was still infiltration with immunoglobulin containing cells. The second case (case 122) developed disseminated intravascular coagulation following an influenza virus infection. On initial biopsy there was IgG, IgA, IgM, C_3 and fibrin in glomerular capillary walls, but one

week later there was only IgG, IgA and fibrin, and at this time there was considerable histological improvement in the underlying disseminated intravascular coagulation. Some three weeks later there was marked resolution of the histological appearances but IgA, IgM and fibrin were still demonstrable within glomerular capillary walls. The third patient (case 141) was a patient with acute tubular necrosis due to leptospirosis. On initial biopsy IgM and C_3 were seen in glomerular capillary walls, but on the return of renal function two weeks later no immunofluorescence could be detected in the glomeruli. The final case (case 231) was a patient who presented post partum with acute renal failure and was found on biopsy to have a rapidly progressive proliferative glomerulonephritis. She made a considerable improvement in renal function and a renal biopsy was performed three weeks later. These biopsies had very similar appearances on histology but by immunofluorescence the first showed fibrin in glomerular capillary walls and crescents, whilst this was not visible in the second specimen. However, this might have been due to the paucity of glomeruli with crescents in that part of the specimen examined by fluorescence microscopy.

In the remaining biopsies there was no significant difference either in the immunofluorescence pattern or the histological appearances between the first and the repeat biopsy. Even in those biopsies performed in transplant patients there was not marked differences between the two specimens, with the exception of Case 26 which was biopsied some three weeks after transplant for primary non-function; at this time the histological appearances were those of an acute tubular necrosis. On immunofluorescence microscopy

there were IgM-containing cells within the interstitium and a fibrinous exudate in the interstitial space. Some ten weeks later a rejection episode appeared and there was widespread glomerular deposition of immunoglobulin, complement and fibrin, and this was reflected by the appearances on light microscopy of an acute rejection episode.

In this study repeat biopsies were performed in twenty-five patients. In no instances apart from transplant patients and patients with acute tubular necrosis was there any significant change in the underlying histological appearances between the first and subsequent biopsy. Those patients with mild proliferative glomerulonephritis who were treated with Indomethacin appeared to show some resolution of the proliferative process and some decrease in their immunofluorescence appearances, although there did not appear to be any marked change in their underlying renal function.

34. MESANGIAL CELLS AND PROLIFERATIVE GLOMERULONEPHRITIS

The distribution of immunoglobulins and fibrin/fibrinogen within the glomeruli of sixty-eight patients with proliferative glomerulonephritis is shown in Table 6 (page

The distribution of immunofluorescence is described as being in the glomerular capillary walls alone, in the mesangial regions alone, or in the walls and mesangial regions. The outcome of the illness is described as being normal if all the abnormal parameters present on initial biopsy had completely resolved, improved if there had been significant improvement in one or all the abnormal parameters, unchanged if the symptoms and signs remained essentially unchanged, and deteriorated if there was evidence of continuing deterioration in renal function or an aggravation of the presenting clinical symptoms. From this it can be seen that of the sixty-eight patients with a proliferative glomerulonephritis, twenty-six returned to normal function, eleven showed significant improvement, twenty-six remained unchanged and nine deteriorated or died.

In those patients in whom immunoglobulins were either absent or confined to the gomerular capillary walls, only three of fifty-four patients showed any deterioration. One of these patients who presented with nephrotic syndrome developed an overwhelming infection whilst on steroid therapy, and it is possible to surmise that without this complication he would have survived. However, five of twenty-six patients in whom immunofluorescence to immunoglobulin deposition was demonstrated in the mesangial region or in capillary walls and mesangial regions deteriorated or died. Only six patients in this group recovered completely normal function. Similar results are obtained with respect

to fibrin deposition. Two patients deteriorated in the group in which fibrin deposition was either absent or confined to glomerular capillary walls. Four of fourteen patients in whom there was fibrin deposition within the mesangial regions alone or the capillary walls and mesangial regions, proceeded to show significant deterioration in renal function. Only two patients in this group returned to normal.

Mesangial cell proliferation and mesangial matrix was assessed on a semi-quantative basis in patients with proliferative glomerulonephritis, focal proliferative glomerulonephritis, mesangial IgG/IgA disease and Henoch-Schonlein syndrome, and related to the degree of proteinuria and hypertension at initial clinical examination (i.e. untreated). There did not appear to be any relationship between mesangial cell proliferation and proteinuria but there was a relationship between proteinuria and mesangial matrix (Table 29). There did not seem to be any relationship between either mesangial cell proliferation or mesangial matrix and hypertension (Table 30).

TABLE 29

MESANGIAL MATRIX AND MESANGIAL CELL PROLIFERATION AND PROTEINURIA

	Proteinuria G/24 hr.	
Cells	0 - 5	> 5
0	7	1
+	57	12
++	13	10
+++	2	0
Matrix		
0	14	1
+	42	10
++	20	4
+++	4	7

0 no proliferation: normal matrix

\+ slight proliferation; slight increase in matrix

++ moderate proliferation; moderate increase in matrix

+++ marked proliferation; marked increase in matrix

TABLE 30

MESANGIAL MATRIX AND MESANGIAL CELL PROLIFERATION AND HYPERTENSION

	Blood Pressure Diastolic, mm Hg	
Cells	<100	>100
0	7	1
+	57	11
++	15	10
+++	2	0
Matrix		
0	11	1
+	107	9
++	19	7
+++	7	5

0 no proliferation; normal matrix

\+ slight proliferation; slight increase in matrix

++ moderate proliferation; moderate increase in matrix

+++ marked proliferation; marked increase in matrix

V DISCUSSION

1. DIFFUSE PROLIFERATIVE GLOMERULONEPHRITIS

Diffuse proliferative glomerulonephritis is the most common type of primary glomerulonephritis but in spite of this classification has provided and is still providing considerable difficulty to pathologists and clinicians alike. The reason for this is not difficult to understand. It is possible to classify this condition on the basis of aetiology, but in most cases no clear aetiology is obvious. An alternative method is to base a classification on clinical presentation, but although certain clinical syndromes are more commonly associated with certain histological patterns, renal biopsy performed in patients with similar symptoms show a great variety of histological lesions (Table 27, p. 209). Even a scheme based on the histological pattern can have disadvantages as there is in many patients a natural history with a changing morphology. For example, a patient may present with an acute nephritis, and on biopsy may have a diffuse exudative proliferative glomerulonephritis with proliferation of mesangial cells, infiltration of polymorphs, endothelial cell swelling and evidence of fibrin deposition. Some time after this active appearance there may be resolution with a disappearance of polymorphs, a subsidence of endothelial cell swelling and only minor proliferation of mesangial cells with slight prominence of mesangial matrix. However, the condition may not resolve and there may be increase in mesangial matrix and focal sclerosis of a variable degree. This has been called progressive

proliferative glomerulonephritis or latent chronic glomerulonephritis. It would probably be most satisfying to classify proliferative glomerulonephritis on the basis of pathogenesis but this is only rarely possible. There is little doubt that proliferative glomerulonephritis is usually immunologically mediated but the immune response evoked, the type, duration and quantity of antigen; the type, duration and quantity of antibody; and the balance between the humeral and cellular responses are all variables which may significantly affect the end result of such a process. Thus proliferative glomerulonephritis is a condition which is difficult to classify on these criteria.

Proliferative glomerulonephritis affects both children and adults. In this series the clinical presentation varied considerably and this is in agreement with most other workers (Habib, 1973, Cameron, 1973). In those patients with diffuse proliferative glomerulonephritis the most common presentation was that of nephrotic syndrome, although asymptomatic proteinuria was a close second. A history of acute nephritis was only found in a quarter of patients with diffuse proliferative glomerulonephritis, but, was present in all patients with a histological finding of exudative proliferative glomerulonephritis. A significant finding appeared to be that those patients who had a progressive lesion on histology presented in a different way from patients with either a mild, moderate or exudative appearance. Three of the eighteen patients in this group presented with hypertension. The reason for this difference in presentation is not clear. It is possible that those patients with progressive proliferative glomerulonephritis had a previous asymptomatic

glomerulonephritis which resulted in hypertension which was then responsible for the progressive nature of the illness. However, it is just as likely that the glomerulonephritis did not resolve satisfactorily and that the hypertension is just an indication of persisting glomerular disease.

The aetiology of the glomerulonephritis was not clear in the majority of patients. It is known that proliferative glomerulonephritis may follow a variety of bacterial or viral infections. In this study only twenty-one of seventy-eight patients gave a history of some infective illness which was followed after a latent period of approximately one to three weeks by an acute nephritic illness. In only twelve patients was there seriological evidence of a preceding streptococcal infection. This may in some instances be due to the difficulty in isolating the streptococcus and also because some strains, particularly type 12, may not produce streptolysin O and thus, even in the presence of true infections, may not be associated with a raised ASO titre (Rammelkamp 1957). In spite of this it would appear that in this study the streptococcus can only occasionally be implicated as a cause of proliferative glomerulonephritis.

The immunofluorescence findings are in agreement with an immunologically mediated disease. The most common finding was that of IgG and complement in capillary walls and mesangial regions. The deposition was in a diffuse granular pattern as described by many other workers (Freidman, Peters and Kark 1960 and McCluskey et al. 1966). Fibrinogen was also detected in a large number of biopsies. Previous studies have indicated this to be usually within the

mesangial region, (Feldman et al. 1966, Morel-Maroger et al. 1972) but in this study localisation within the mesangial regions was rare. The most common localisation was within capillary walls alone (26 cases) while in a further 14 it was within capillary walls and mesangial regions. In only three cases, all with exudative proliferative glomerulonephritis, was the fibrin deposition defined to the mesangium. The reason for this discrepancy is not clear. In conditions other than proliferative glomerulonephritis I have similarly found a paucity of fibrin deposition in the mesangium. I have assumed this to be in some way related to the dynamic nature of fibrin deposition and subsequent removal by fibrinolysis but it may also be due to differences in the antisera employed to detect fibrin deposition. Another interesting finding is the frequent deposition of IgM which is contrary to many other reports. In this study IgM is found as frequently as IgG in diffuse proliferative glomerulonephritis. This is an unusual finding in as much as the majority of patients were biopsied late in their disease. IgM is considered to be produced in greatest quantities early in the immune response after which its concentration tends to decline as IgG becomes the predominant immunoglobulin. In view of this one would expect to find IgM only at an early stage of the disease and not with the frequency seen in this study. The reasons for this unusual finding are not apparent.

In a number of cases studied there has been no specific immunofluorescence detected within the biopsy. It is possible that the biopsy has been carried out at a stage in the disease process where the immune complexes and products of inflammation have been adequately removed but there are persisting urinary and histological

abnormalities. On the other hand it is possible that material deposited within the glomerulus is in a form unsuitable for examination by immunofluorescence techniques or that the amounts deposited are insufficient for detection by this method.

A search for specific antigens was not undertaken in this study.

The findings of immunoglobulin, complement and fibrin within the glomeruli with patients with proliferative glomerulonephritis supports the concept of an immunologically mediated disease. The pattern of deposition suggests an immune complex mediated condition and this is in agreement with most of the experimental studies of this condition. Germuth (1953) showed that a diffuse proliferative lesion developed following the injection of bovine serum albumin into rabbits and that elimination of the antigen was associated with a regression of the glomerulonephritis. It was suggested that antigen antibody complexes form and these become localised within the glomeruli. This was further confirmed by McCluskey et al. 1960 who produced a proliferative glomerulonephritis by injecting complexes into mice. Fish et al. 1966 demonstrated antigen, antibody and complement by immunofluorescent methods in the glomeruli of rabbits injected with bovine serum albumin. There is thus plenty of evidence, both experimental and clinical, to support the concept of proliferative glomerulonephritis being an immunologically induced disease.

The clinical course of diffuse proliferative glomerulonephritis is variable with some patients returning to normal over a variable period of time and others progressing to end stage renal failure. The age of the patient is of some importance. In the present study the older patients appeared to have more persisting disease and a higher

incidence of progressive renal impairment. This is similar to other studies which report, more than 80 per cent of children recovering (Lieberman and Donnell 1965, McCrory and Shibuya 1968) and less than 70 per cent recovery in adults (McCluskey and Baldwin 1963). Long term follow-up is required in any study as resolution may occur more than two years after the onset of the disease. In the present study patients have been followed for up to four years from the time of biopsy. The natural history is difficult to define as, with the exception of acute nephritis, it is not possible to know how long the pathological process was present prior to the patient becoming symptomatic. The considerable number of patients who are found to have asymptomatic proteinuria, almost 25 per cent in the present series, suggests that glomerulonephritis may be present for some time prior to becoming clinically manifest. It is also possible that some patients may never develop symptoms at any time in the course of the disease.

Hypertension appears to be a factor indicating poor prognosis (Table 7, p. 98). The reason for this is not clear. It is possible that the hypertension reflects the severity of the glomerular injury, marked glomerulonephritis producing renal ischaemia and thus stimulating the renin-angiotensin system resulting in hypertension. However against this is the fact that in rapidly progressive (crescentic) glomerulonephritis, where there is severe glomerular damage, hypertension is not common. It is possible that the extent of the glomerular injury is critical with minor changes having no effect and severe changes also having little effect. However the hypertension may not be causally related to the glomerular disease and it might be that

glomerulonephritis occurring in a patient with pre-existing hypertension has an adverse effect on the natural history of the condition. It is rare to have adequate records of blood pressure prior to the development of renal disease.

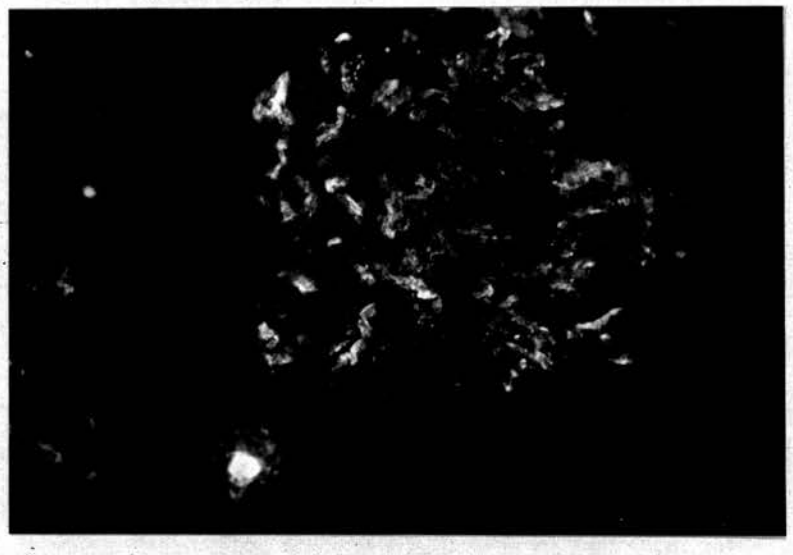
The histological findings are of some value in determining the prognosis in patients (Table 8, p. 98). The finding of irregular increased mesangial matrix producing hyalinised areas is of poor prognosis. Presumably in such cases there is a slow steady expansion of the sclerotic area until the glomerulus is completely hyalinised. This occurring in a significant number of glomeruli will result in a progressive irreversible reduction in renal function. Exudative lesions produce a variable prognosis presumably due to factors such as the duration of antigen and the quantity and quality of antibody produced. The exudative lesion is only a manifestation of inflammation and providing the initiating event can be contained then the inflammatory response will subside and the condition resolve.

The immunofluorescence findings appear to be of value in assessing the prognosis of proliferative glomerulonephritis (Table 6, p. 97). Those patients with no immunofluorescence detected to immunoglobulins, complement or fibrin probably have only a mild illness with only a small amount of inflammatory components becoming deposited in the glomerulus. In moderately severe disease most of the abnormal deposits are in capillary walls although there is evidence of some in the mesangium (Fig. 97). In other patients large amounts of material is visible in glomerular capillary walls and mesangium (Fig. 98) giving the impression that the rate of deposition of material in the glomerulus exceeds the capacity for

removal. The prognosis of patients in this group is poor. The phagocytic capacity of the mesangial cell has been demonstrated to a wide variety of materials (Garquhar and Palade 1962, Michael, Fish and Good 1967), and it has been suggested that an important function of the mesangial cell has been to remove material deposited in the glomerular capillary wall. Material which is deposited in the capillary wall can only be removed by lysis and absorption into the systemic circulation, phagocytosis by circulating cells, passage through the capillary wall into the urinary space and phagocytosis by the mesangial cells. There is evidence that in glomerulonephritis all these four mechanisms are operative. It is known that in glomerulonephritis there is increased plasma and urinary fibrin degradation products indicating lysis and excretion of deposited fibrin. In certain forms of experimental glomerulonephritis infiltration of mononuclear phagocytic cells has been demonstrated (Shigematsu and Kobayashi 1971) but their role of human glomerulonephritis is unknown. The mesangial cells are capable of removing material from the subendothelial region of the glomerular capillary wall (Farquhar and Palade 1962, Kelly 1970). They are able to ingest colloidal material (Mellors and Brzoskol 1961) and aggregated gammaglobulin (Michael et al. 1967). The ultimate fate of immunoproteins or complexes once they have been taken up by the mesangial cells is unknown. It is possible that the material is transmitted through the mesangium to the hilum of the glomerulus and thence to the distal tubule, the efferent arteriole or the renal lymphatics. The outcome of an episode of proliferative glomerulonephritis may thus depend on the ability of the mesangium to deal

with the products of inflammation. Progressive disease would result when the amount or size of material was such that it could not be removed through the mesangium.

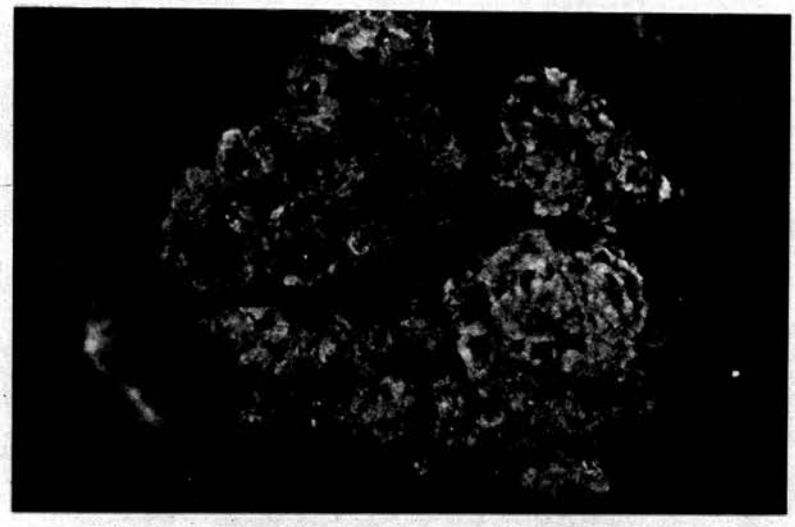
Figure 97



Immunofluorescence to IgG in a patient with diffuse proliferative glomerulonephritis. The IgG is localised to capillary walls although some is also present in mesangial regions. There are a number of capillary walls free from deposit. This patient had a satisfactory resolution of his illness.

Case 212 IgG x 250

Figure 98



Immunofluorescence to complement (C_3) in a patient with diffuse proliferative glomerulonephritis and progressive impairment of renal function. There are large deposits of material in all capillary walls and mesangial regions.

Case 126 Complement (C_3) x 300

2. RAPIDLY PROGRESSIVE (CRESCENTIC) GLOMERULONEPHRITIS

It has been recognised for many years that some patients with glomerulonephritis present with an acute syndrome and then pursue a rapid course to death in uraemia in a few weeks or months. This form of glomerulonephritis has been given many names. Ellis (1942) called this "rapidly progressive type 1 glomerulonephritis", whilst it had been previously described by Volhard and Fahr in 1914 as extra-capillary glomerulonephritis. More recently it has been described as acute anuric glomerulonephritis (Berlyne and Baker 1964), glomerulonephritis with crescents (Brewer 1964), and rapidly progressive (non-streptococcal) glomerulonephritis (Bacani et al. 1968). The term rapidly progressive glomerulonephritis is now used for a syndrome which is characterised by an acute onset with oliguria and anuria and in which the renal biopsy reveals large circumferential crescents in many glomeruli (more than 70%) associated with little or no mesangial cell proliferation. A similar "rapidly progressive" syndrome may be found in other conditions such as Goodpasture's Syndrome, Henoch-Schonlein disease, microscopic polyarteritis and some cases of post-streptococcal glomerulonephritis. The differentiation of rapidly progressive glomerulonephritis from these conditions can on occasions be difficult and is usually made on clinical criteria or on laboratory evidence such as the detection of anti-GBM antibodies or a raised ASO titre.

This condition can occur at any age and whilst the average age of patients in this study was 56 years this is considerably older than those cases described by Davson and Platt (1949)., Harrison et al. (1964) and Heptinstall (1974a). However, most authors would agree that this is a condition of adults and it is not fair to draw

any true conclusion regarding the age incidence from a series of only five patients. In most other series, however, there does seem to be a male/female ratio of approximately 2 : 1 whereas in the present series there are three females and two males. Again it is likely that the small number in this series accounts for this discrepancy. It is interesting to note that Striker et al. (1973) states that there is no clear cut predilection for either age or sex.

The immunofluorescence findings in this series is in broad agreement with that of other reports (Burkholder et al. 1969, Berger et al. 1971, Lewis et al. 1971 and Morel-Maroger et al. 1972). Immunoglobulins are detected within the glomerular capillary wall with a granular distribution. The most common immunoglobulin is IgG whilst in two cases IgM is also present. In no case was IgM present in the absence of IgG. No IgA was present in this series and this is also in agreement with previous reports. Complement (C_3) was only detected in two of the five patients. Morel-Maroger et. al. (1972) found complement (C_3) without immunoglobulin but this pattern has not been seen in this present study. Lewis et al. (1971) detected C_3 component of complement but no Clq or C_4. Likewise in the present study we have not detected any deposition of C_4. This could be on account of the fact that the sub-group of IgG deposited does not fix complement and this may also explain the paucity of polymorphs and proliferative activity. The most common finding in our series has been the demonstration of fibrin within epithelial crescents. This is similar to previous reports (Bacani et al. 1968, Koffler and Paronetto 1965 and Lewis et al. 1971). In no patients have we detected a linear deposition of immunoglobulins in the glomerular capillary wall. This

is in contradiction to the findings of Heptinstall (1974) who frequently found a linear deposition in such patients. This may be explained by the fact that patients with circulating anti-GBM antibody which becomes deposited in glomeruli to develop a renal disease, associated with haemorrhagic lung disease (Goodpasture's Syndrome) which show a linear deposition on glomerular basement membrane but we have only one such patient in our study.

The aetiology and pathogenesis of this condition is unknown. It has been suggested that there is an immune mediated injury to the glomerular capillary wall and that this so alters the permeability of the wall that fibrinogen is able to enter Bowman's space. This is followed by the deposition of fibrin causing proliferation of parietal epithelial cells with subsequent crescent formation. In this study a consistent finding has been the detection of fibrin in the characteristic circumferential crescents but it is not possible to state whether the fibrin is causally related to the crescent or appears after its formation.

The lack of marked mesangial cell reaction is of considerable interest. In these patients it is frequently difficult to identify mesangial cells on light or electron microscopy. There thus appears to be a state of mesangiolysis but again it is impossible to know whether this is cause or effect. It might be that in patients with mesangial impairment there is alteration to capillary wall permeability resulting in fibrinogen exudation and crescent formation. However, it is just as possible that the immunological insult is of such severity that there is a direct 'toxic' effect on the mesangial cell producing cell death and apotosis.

Beaufils et. al. (1976) described four patients with visceral abscesses who developed rapidly progressive glomerulonephritis with acute renal failure and they suggested that severe infections may be responsible for rapidly progressive glomerulonephritis although they did not enlarge on the potential pathogenesis apart from indicating that it was probably immune complex mediated. In this study there was no evidence of abscess formation in any patient.

The prognosis of patients with this condition is uniformly bad. The vast majority progress to terminal renal failure either requiring haemodialysis or transplantation within some months of clinical presentation. In our group one patient has improved; while this is not unknown it is certainly uncommon. In a series of 63 patients studied by Striker et al. (1973) only four patients returned to normal, whilst 52 failed to recover any renal function at all.

3. MEMBRANOUS GLOMERULONEPHRITIS

Membranous glomerulonephritis is characterised by a widespread uniform thickening of glomerular capillary walls. It has been termed membranous nephropathy (Ehrenreich and Churg 1968), perimembranous glomerulonephritis (Bohle 1964), epimembranous nephropathy (White 1969) and extramembranous glomerulonephritis (Berger 1961). The term membranous glomerulonephritis was first introduced by Bell (1950) and this was assumed to be the pathological lesion associated with Ellis Type 2 nephritis (Ellis 1942). Jones (1957), employing silver stains, showed that the thickening of the basement membrane was not uniform but consisted of projections of argyrophilic material from the outer aspect of the basement membrane. Between these small "spikes" there were foci which did not take up the silver stain. This appearance was also described by Churg and Grishman (1957), using the PAS stain. Following the widespread introduction of renal biopsy Berger (1961) demonstrated that the areas devoid of silver staining were indeed subepithelial deposits.

Diffuse membrane thickening on light microscopy examination is not confined to idiopathic membranous glomerulonephritis. It may be seen also in patients with systemic lupus erythematosus, diabetes mellitus and in some cases of renal transplantation. The term idiopathic membranous glomerulonephritis should be reserved for those patients who present with a typical history, a histopathological appearance with "spikes" of silver staining, a granular immunofluorescence pattern and subepithelial electron dense deposits on electron microscopy.

The clinical presentation of patients with this condition is

typically that of the nephrotic syndrome (Pollak et al 1968). In the present study of sixteen patients some fourteen presented with nephrotic syndrome and two were detected at the stage of asymptomatic proteinuria. This therefore is in close agreement with most other reports. The number of patients presenting with asymptomatic proteinuria will vary depending very much upon the population studied and their mode of referral. In a series reported by Habib and Kleinknecht (1973), twenty-seven of fifty children were detected with asymptomatic proteinuria; this high incidence is probably due to the routine urine testing of French school children. The average age of onset in the present study is forty-eight but there is a wide age scatter from twenty-two to seventy-four years. No children in the patients reported here had this disease and this may represent a true geographical difference in the incidence of the condition or reflect the relatively small number of children examined in this study. Although membranous glomerulonephritis accounts for only some 13% of adults with primary renal disease in this group it accounts for 30% of adults presenting with nephrotic syndrome. This is in close agreement with the findings of Cameron (1968) who found that membranous glomerulonephritis accounted for 39% of nephrotic syndrome in adults with primary glomerular disease. An unusual finding in the present study is the high male incidence. Most reported series report approximately a 60% male incidence but in this study there are thirteen male patients and only three female. In all other respects the present group appear fairly typical in as much as there is no history of precipitating or associated events such as respiratory tract infections or other bacterial or viral illnesses. Malignancy

is associated with a membranous lesion (Loughridge and Lewis 1971, Higgins et al. 1974) but no patients in the present series had any evidence of an associated malignancy and none developed during the one to four years of follow-up.

Immunofluorescence studies have been performed on many biopsy specimens and fairly consistent results have been obtained. The most common finding is of a diffuse granular type of fluorescence along the glomerular capillary walls to IgG and complement (C_3). In this study the amount of IgG deposition appeared to be greatest between 12 and 30 months following the onset of symptoms. It is also interesting to note that after 72 months from the onset of symptoms no immunofluorescence was detected in the five patients studied. IgM and IgA were only rarely encountered and when present, usually only in small amounts. Eleven patients were studied for the presence of C_4 component of complement, and this was positive in four of such patients suggesting a classical pathway activation of complement in this condition. Fibrin deposition in a subendothelial position was a common finding, but again was restricted to patients examined only in the first four years of symptomatic disease.

The reason for the absence of specific immunofluorescence in those patients examined five or more years after the initiation of their symptoms is not at all clear. Negative immunofluorescence in patients with membranous glomerulonephritis has been previously reported; Bariety et al. (1968) described one patient whose immunofluorescence was negative in association with a clinical remission. Unfortunately there is no indication of the time between presenting symptoms and the biopsy. Similarly Murphy et al. (1973)

described negative immunofluorescence in four of their 39 patients. Unfortunately two of these patients had been treated with steroids and again it was not clear how long had elapsed between the onset of symptoms and the biopsy. It is possible that immune complexes once deposited in a subepithelial position are gradually removed in time and that the immunofluorescence will become negative providing that antigenic stimulus is removed and the immune complex deposition ceases. Alternatively it is possible that the antigenic sites on the complexes are covered and therefore not available for binding with the fluorescein conjugated antiserum. Unlike the patient of Bariety et al. (1968) the five patients in this study with negative immunofluorescence were not in clinical remission. One was lost to follow up but of the remaining four two died from chronic renal failure, one is progressing with increasing renal impairment and one remains unchanged. Unfortunately in this study we have only examined one patient by immunofluorescence on two occasions but the time interval was only twelve months between biopsies and identical findings were obtained. It will be important to re-examine those immunofluorescence positive patients in the coming years to see whether the immunofluorescence diminishes and/or changes with time.

The clinical outlook in this condition appears to be very variable. The method of clinical presentation is of no prognostic value because there is such a high preponderance of patients presenting with nephrotic syndrome. It would appear that the degree of proteinuria gives no indication as to the probability of development of renal failure. Similarly hypertension appears to be

of little prognostic value. In a series studied by Franklin et al (1973) five of eight patients who had a diastolic pressure greater than 90 mm Hg progressed to renal failure. However, nine of the twenty-five who were normotensive at initial presentation also progressed to renal failure. In the present study seven of fourteen patients had a diastolic pressure greater than 90 mm Hg and this was not related either to the creatinine clearance or to their subsequent prognosis. The only significant finding appeared to be that of the five patients who were aged 40 or less at onset, three are dead from chronic renal failure, while of those who were over 40 only one of twelve has died from chronic renal failure. This, of course, is contrary to the findings of Habib et al. (1973) who found that in their young patients 26 of 50 patients went into remission. However this was a study in children and may bear little relationship to the disease process in adults.

The findings of IgG and complement in capillary walls and associated with dark granular subepithelial hump-like deposits on electron microscopy is very suggestive of an immune complex mediated disease. However, it is also known that immune complexes play a significant part in the pathogenesis of proliferative glomerulonephritis. Clearly there must be some difference between the immune complexes in membranous glomerulonephritis and those in proliferative glomerulonephritis. The localisation of complexes in a subepithelial position is difficult to explain. It is known that in the mouse ferritin-antiferritin complexes pass through the basement membrane and appears to accumulate in a subepithelial position particularly at the junction between podocytes (Kelly and Cotran 1972). This might represent a

true filtration barrier to material of a particular size, shape or electrical charge. However, more recently receptor sites to altered third component of complement (C_3b) have been demonstrated within the glomerulus (Gelfand et al. 1975) and it has been suggested that these bind antigen-antibody-complexes. It has been also suggested that these receptors are sited in the epithelial cells (Burkholder et al. 1977) and if this is the case it could explain the typical deposition seen in membranous glomerulonephritis.

Germuth et al. (1967) found on repeated injections of bovine serum albumin into experimental animals that this resulted in a membranous glomerulonephritis when a repeated low dose schedule was employed. A similar finding was reported by Dixon et al. (1961) who found that intravenous injections of small amounts of heterologous protein into rabbits produced a membranous glomerulonephritis if the antibody response was low and that the antibody produced combined with antigen in a relative antigen excess. This would seem to suggest that repeated formation of small amounts of soluble antigen-excess complexes will lead to a membranous glomerulonephritis, and this is supported by the presence of membranous glomerulonephritis in certain other immune complex mediated clinical situations. For instance, a membranous glomerulonephritis has been reported in patients with syphilis (Braunstein 1970), filariasis (Bariety et al. 1967) and quartan malaria (Hendrickse et al. 1972). Each of these conditions is associated with antigen persisting for a long period of time. It may therefore be that those conditions with a persisting antigen, particularly if it is intracellular, give rise to small repeated liberations of antigen into a circulation which has a low

concentration of antibody, therefore leading to repeated episodes of deposition of soluble immune complexes. Alternatively it may represent some deficiency of cell mediated immune response in the patient. This is supported by the case report of Kohler et al. (1974) who described a patient with hepatitis B antigen associated membranous glomerulonephritis. This patient was unable to amount a cell mediated immune response to the hepatitis antigen and it is thought that this failure of cell mediated immune response was responsible for the persistence of the antigen and therefore the continual production of antibody. It may therefore be that with further study the aetiology of membranous glomerulonephritis will be found to include an element of immunological deficiency.

4. MESANGIOCAPILLARY GLOMERULONEPHRITIS

There has been considerable discussion over the past ten years as to the nomenclature of this disease. Royer et al. (1962) described three cases of a chronic glomerulonephritis associated with mesangial cell proliferation and a considerable diffuse thickening of the glomerular capillary walls. This condition was termed membrano-proliferative glomerulonephritis. Since then the disease has been called persistent or chronic hypocomplementaemic glomerulonephritis (West et al. 1965, Herdman et al. 1970), membrano-proliferative glomerulonephritis with hypocomplementaemia (Michael et al. 1969), mesangiocapillary glomerulonephritis (Churg et al. 1970), mixed membranous and proliferative glomerulonephritis (Burkholder et al. 1970), membranous proliferative glomerulonephritis (Zolinger 1971) and chronic mesangioproliferative glomerulonephritis (Mandalenakis et al. 1971). Lobular glomerulonephritis is another term which has been used for this condition although there is considerable debate as to whether this histological appearance represents a distinct entity or a late stage of mesangiocapillary glomerulonephritis. Electron microscopy reveals two distinct types of mesangiocapillary glomerulonephritis. In the first there is a focal accumulation of material deposited in the subendothelial region and this has been termed "subendothelial" type disease, whilst in the second there is an irregular thickening of the basement membrane which is intensely osmiophilic and was first described by Berger and Galle (1963); this is called "dense deposit" disease. In this study the term mesangiocapillary glomerulonephritis has been used for both subendothelial type disease and dense deposit type disease.

Characteristically this is a disease of children and young adults as approximately two-thirds of cases become manifest before the age of 20 (Cameron et al. 1970). There is, however, an extremely wide scatter as indicated by the age range in this study. It affects both sexes equally although there is a male preponderance in the present series. The clinical presentation is variable, about one-third presenting with acute nephritis and one-third as nephrotic syndrome. In about 50 per cent of cases there appears to be a preceding acute infection (Lagrue et al. 1973) and a recent study (Jenis et al. 1974) report a seriologically proven streptococcal infection in approximately 20 per cent of cases. In the present series three of thirteen patients had an acute infection prior to their nephritis becoming manifest but in no case was there evidence of a streptococcal infection. In no instance was there any evidence of familial disease and the past history was unremarkable in all cases. Hypertension is common in this condition and although it may be of a transient nature in approximately 15 per cent of cases it will re-appear within five years of the onset of the disease. (Lagrue et al. 1973). The incidence of hypertension in the present study was slightly greater than that in other reported series and the transient hypertension previously described has not been observed.

Proteinuria is common in this condition and characterically it is heavy and of a non-selective character. Haematuria invariably occurs at some time during the illness. Impaired renal function is common with a reduced creatinine clearance being found in about half of the cases and some 40 per cent showing signs of renal insufficiency at the onset of their illness. (Habib et al. 1975). In the present

study eight of thirteen patients had a creatinine clearance of less than 80 mls per minute at the time of their biopsy.

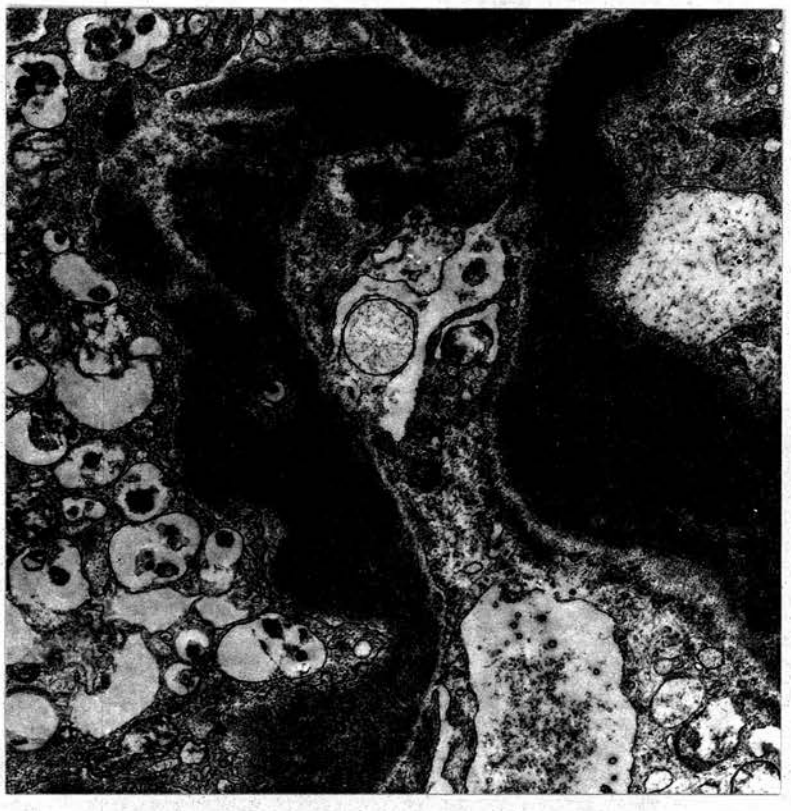
The immunofluorescence findings have been fairly constant in the reports of many workers (Berger et al. 1971, Herdman et al. 1970 and Morel-Maroger et al. 1972). The most common finding is a granular deposition of complement along the capillary walls particularly at the periphery of lobules. In the present study it was detected in six of thirteen patients. This is in contrast to the much higher incidence of other workers (Westberg et al. 1971, Michael et al. 1971, Michael and McLean 1974, Davis and Cavallo 1976) who all found complement (C_3) in all biopsies examined in their respective series. It has been suggested that immunofluorescence diminishes with time from onset of symptoms (Herdman et al. 1970) but this would not be supported in the present series nor many other series and so this cannot be the explanation for the disparity in findings. Immunoglobulins, particularly IgG and IgM are distributed with a pattern similar to that of complement and are present in approximately 60 per cent of cases. IgA has only rarely been detected with the exception of the one reported series (Pollak et al. 1973) where it was seen in fourteen of sixteen cases. Towards the end of the present study antiserum to complement (C_4) became available and deposition of C_4 was detected in two of the four biopsies examined. There have been few previous studies of complement (C_4) deposition in mesangiocapillary glomerulonephritis but it was found in sixteen of the sixteen biopsies examined by Davis and Cavallo (1976).

The prognosis in this condition is poor. In the present study some five patients have deteriorated or died whilst only one has

shown any sign of improvement. This is essentially the same as that reported by Cameron et al. (1970) where 15 of the 31 patients deteriorated or died. However, a considerable number of patients appear to remain in stable renal function although obviously in a condition which can run a prolonged clinical course the overall prognosis must be guarded. The immunofluorescence findings give no indication as to the prognosis in any individual patient.

Partial lipodystrophy is a rare condition of obscure aetiology, occurring mainly in children and young adults and with a marked female preponderance. It is characterised by a symmetrical progressive and painless loss of subcutaneous fat usually commencing in the face and scalp and progressive caudally as far as the iliac crests. In some instances there is increased adiposity in the lower limbs. Hypertension is common (Eisinger et al. 1972) and impaired carbohydrate tolerance and frank diabetes mellitus may occasionally occur. Mesangiocapillary glomerulonephritis of the dense deposit type (Fig. 99) frequently occurs in patients with partial lipodystrophy. The latent period before the glomerulonephritis becomes manifest is variable and may be as long as 20 years. The two patients in the present series are very similar to those reported by other workers, (Eisinger et al. 1972, Williams et al. 1972 and Peters et al. 1973). Unfortunately during the present series complement studies were not available and therefore no comment can be made regarding hypocomplementaemia and nephritic factor in mesangiocapillary glomerulonephritis.

Figure 99



Electronmicrograph of a dense deposit type mesangiocapillary glomerulonephritis in a patient with partial lipodystrophy.

5. FOCAL PROLIFERATIVE GLOMERULONEPHRITIS

In focal proliferative glomerulonephritis the lesions on light microscopy appear to have a focal distribution, only some glomeruli being involved while others appear normal (see II.4.f). In addition, in the affected glomeruli only some segments exhibit proliferation while the remainder of the glomerulus is normal. If only small parts of a glomeruli are involved it is clearly impossible to be sure that every glomerulus is not affected, since the section examined may have been cut in such a way as to exclude the abnormal part. To be certain that a glomerulus was normal would require serial sections of the entire biopsy with very careful examination of every whole glomerulus contained in it. I have not seen such a study reported and it was not carried out in the present series.

There are several conditions, such as Henoch-Schonlein purpura, subacute bacterial endocarditis, polyarteritis and systemic lupus erythematosus, which may have predominantly focal glomerular lesions, but in this study such cases have not been classified simply as focal proliferative glomerulonephritis, as all clinical findings and laboratory investigations have been taken into consideration when reaching a final diagnosis.

The patients in this study appear to differ in several ways from other reported series. Most authors record the clinical presentation as recurrent haematuria (Bates et al. 1957, Heptinstall and Jocker 1959, Ross 1960) but no patient in this series had such a presentation. However, it must be remembered that the above reports were specifically of biopsy findings in patients with recurrent haematuria, whilst in this series patients were not selected in this way. Hypertension is

not a feature of other series, but was present in three of the eleven patients studied. The prognosis and response to steroids is said to be good in these cases (West et al. 1968); the present group would not support this claim but it must be remembered that the patients described by West and his colleagues were children, in contract to the adults in the present study.

The immunofluorescence findings in focal proliferative glomerulonephritis are interesting in as much as the distribution of immunoglobulins, complement or fibrin/fibrinogen is diffuse and does not reflect the focal nature of the disease as seen on light microscopy. This is in agreement with other workers. It is not clear why there should be this disparity between the immunofluorescence findings and the light microscopy. It is impossible to believe that the nature of the deposited material differs from one part of the glomerulus to another or from one glomerulus to another. However, it is possible that the intraglomerular and intrarenal blood flow distribution may have some part to play in the amount of material deposited and the rate of accumulation in a particular part of the glomerulus. Local factors such as mesangial response are probably of importance, but as yet there is no clear indication of the nature of these suggested local factors. The predominant immunoglobulin in this series is IgM, while that of the series reported by Berger and Hinglais (1968) is IgA. There are few reports of immunofluorescence in focal glomerulonephritis and so it is not possible to do other than draw attention to this disparity. The patients of Berger and Hinglais appear somewhat different from those of the present series in as much as they tended to be young adults with recurrent haematuria and seemed to form

a fairly homogeneous group. The patients in the present study are, however, remarkably heterogenous with respect to age, clinical findings and prognosis.

The aetiology of this condition is not clear. It is possible that there is no single aetiological factor but that it represents an unusual glomerular response to a wide variety of conditions. It does not appear to be related to streptococcal infection, as there was no significant history of preceding infection and the ASO titre was not elevated in any of the cases. However, with the finding of IgM as the major immunoglobulin there may be some relation to complex size or defect in the production of IgG in response to antigenic stimulation. This condition therefore presents many problems which will become clarified only with further study.

6. MESANGIAL IgG/IgA DISEASE

This condition was first reported by Berger and Hinglais (1968) when they documented a series of twenty-five patients whose renal biopsies, by immunofluorescence techniques, showed marked deposition of IgA in the mesangium of glomeruli, associated with less intense staining with IgG and C_3. On light microscopy there was a prominence of mesangial regions and a mild proliferation of mesangial cells. Since this report there have been many other essentially similar reports (Berger 1969, deWerra et al. 1973, Druet et al. 1970, Hyman et al. 1973, Lowance et al. 1973, Morel-Maroger et al. 1972). Although there has been considerable discussion as to whether mesangial IgG/IgA disease represents a distinct disease entity it is now generally accepted that it describes a condition which is more common in males than females, occurs in older children or young adults, presents as either recurrent haematuria or asymptomatic haematuria found at routine medical examination, is infrequently associated with hypertension and rarely causes renal failure. The light microscopic appearances are variable; in some cases the appearances are virtually normal whilst in others there may be a variable prominence of the mesangium or a focal proliferative glomerulonephritis which may even show focal necrotising changes. On immunofluorescence microscopy the most obvious feature is a bright staining in the mesangium with IgA. This may be associated with some IgG and complement (C_3) deposition. On electron microscopy deposits can be seen within the mesangium and occasionally in a sub-endothelial position.

The two patients in this series fit well with the above description.

They are both males, both presented with recurrent haematuria, and both have only slight proteinuria. The unusual feature is that the blood pressure in these two patients is moderately elevated being 160/98 in case 43 and 115/90 in case 225 who is a six year old boy. Although proteinuria is usually minimal, Druet et al. (1970) reported fifty-two patients of whom seven had a protein excretion exceeding 3 grams per 24 hours; in the series of deWerra et al. (1973) three of ninety-six patients had a similar protein excretion. Case 43 in this series had proteinuria to the extent of 3.1 grams per 24 hours.

The incidence of this condition is not known. In view of the fact that the patient may have only microscopic haematuria the incidence in any series will depend upon the population studied. Berger (1969) described this condition in fifty-five of three hundred biopsies. However, since then all other series have described a lower incidence.

Berger 1969	18%
Morel-Maroger et al. 1973	7.7%
Doyle et al. 1976	6.2%
McCoy et al. 1974	4.3%
Sissons et al. 1975	4%
Hyman et al. 1973	2.1%
Finlayson et al. 1975	2%

In the present series it was present in only 2 of 271 initial biopsies, an incidence of less than 1%.

The reason for the wide range of incidence could well reflect the population studied or the method used to detect the condition. In France many young people are subject to routine medical examinations

which include urinanalysis and as this condition affects predominately young people and may be asymptomatic this may account for the higher incidence reported in French series. It is also possible that there may be differences in technique between centres which renders direct comparison invalid. An important difference between groups is in the antiserum used to detect the presence of IgA. The French group tend to use goat antiserum (Hyland) while Doyle et al. (1976) used monospecific antiserum obtained from Nordic Diagnostics. The present study used an antiserum raised in rabbits and supplied by Hoechst Pharmaceuticals. It is possible that there is considerable variability in different antiserum but I know of no reported series comparing one commercial preparation with another. As the diagnosis of this condition is dependent upon immunofluorescence I feel that the antiserum used is of prime importance, a fact that has been largely ignored.

The aetiology of this condition is unknown. Berger (1968) drew attention to the fact that many of these patients presented with recurrent haematuria in close association with an upper respiratory tract infection and that the tonsils contain numerous IgG and IgA secreting plasma cells. However, McCoy et al. 1974 evaluated their biopsy specimens for the presence of the secretory component of IgA to determine whether this immunoglobulin might comprise the antibody in an immune complex. In only two of the fifteen biopsies studied was there any evidence of the IgA secretory component and thus they suggest that there is no evidence that secretory IgA plays a significant role in the pathogenesis of the condition.

IgA is not believed to fix complement components by the classical pathway and the finding of IgA and complement (C_3) in the glomeruli of

most patients with this condition raises the question of the relationship between these components. It is possible that the alternate pathway is activated in patients with this condition. Only one of the patients in this series was studied with anti-serum to C_4 but it is of interest to note that this was negative although C_3 was present. This would support the concept of complement activation by the alternate pathway but clearly no conclusions can be drawn from one result. It is known that aggregated IgA will fix complement in vitro (Gotze and Muller-Eberhard 1971) but there is no evidence that IgA activates the alternate complement pathway in vivo. However it is possible that enzymatic fragments of C_3 generated during the activation of the classical complement sequence may activate the alternate pathway as described by Muller-Eberhard and Gotze (1972). However it is also possible that the alternate pathway is activated via the properdin system, and this is supported by the demonstration of properdin in fifteen of the sixteen patients studied by McCoy et al. (1974). A further possible explanation is that the IgA containing immune complexes may be of a size that is preferentially deposited within the mesangium. It is known, for instance, that experimental animals with a sustained low level antibody response have circulating complexes with molecular weights of five hundred thousand to seven hundred thousand daltons and these immune complexes are deposited primarily in the capillary wall and cause a diffuse glomerulonephritis. In animals whose circulating complexes have a molecular weight in excess of one million daltons have immune complex deposition primarily in the mesangium, and not in the glomerular capillary wall. Thus it is possible that the site of deposition of immune complexes is a function

of their size and that this will determine to a large extent the glomerular response and ultra-structural appearance.

7. MINIMAL LESION GLOMERULONEPHRITIS

This condition has been known by a variety of names. Munk (1916) introduced the term lipoid nephrosis in view of the finding of fatty bodies in the urine associated with fatty changes in the tubular cells and an absence of histological glomerular changes. Following the introduction of renal biopsy this condition came to be known as minimal change glomerulonephritis, glomerular epithelial disease or foot process type disease, due to the fact that on light microscopy there were no significant abnormalities but on electron microscopy changes in the glomerular epithelial cells could be seen. However, although the definition "the nephrotic syndrome associated with the finding of normal or virtually normal glomeruli by light microscopy" (Pollak et al. 1968) provides a useful working definition it does not take into account the fact that some patients may show minor mesangial changes. The definition of this disease is therefore extremely difficult (Jao et al. 1973, Heptinstall 1974). In this study the term has been used solely for those patients who have proteinuria unassociated with either hypertension or haematuria, and who respond with a remission in the proteinuria following steroid therapy. In addition to this clinical appearance the light microscopy must be either normal or show only minor increases in mesangial cells and/or matrix.

The mean age of the patients in this group (15.4 years) is slightly older than that of most published series. This is probably because many children who present with highly selective proteinuria and the nephrotic syndrome are presumed to have a diagnosis of minimal lesion and therefore not biopsied, and to the fact that in this series

there is a bias towards adult patients. Two of the patients in this group were over the age of 20; while minimal lesion glomerulonephritis clasically occurs in children it is not unknown in adults (Cameron, Ogg and White 1974, Hopper et al. 1970). It is interesting to note that although this is a small series of 20 patients the sex incidence agrees with that of larger published series (Barnett, Forman and Lauson 1952, White, Glasgow and Mills 1970, Habib and Kleinknecht 1971).

The light microscopy in this group of patients shows that in ten it was entirely normal whilst in fourteen there was a slight increase in the mesangial cell matrix. This emphasises the difficulty in distinguishing minimal lesion glomerulonephritis from a mild proliferative or a resolving proliferative glomerulonephritis on light microscopy alone. It is therefore important in reaching such a diagnosis to consider not only the light microscopy but the clinical presentation, laboratory findings and, where known, the response to steroid therapy. Electron microscopy can be of considerable help in establishing the diagnosis. Classically there is loss of pedicel structure with a "smearing" of the epithelial cell on the outer aspect of the basement membrane. This appearance may occur in a patchy fashion in conditions such as proliferative glomerulonephritis, but in minimal lesion glomerulonephritis the pedicel loss is more marked and sometimes almost complete; it is associated with only minor mesangial cell proliferation.

The immunofluorescence findings have been reported in many series. In most of these, immunofluorescence has been negative (Berger et al. 1971, Drummond et al. 1966, Morel-Maroger et al. 1972). Immunoglobulins and complement have been described (Kobayashi 1966 and

Lange et al. 1966) and Drummond et al. (1966) has described a focal deposition of IgG and complement (C_3) in mesangial regions. In these reports the amounts of immunoglobulins and complement deposited have always been small. Drummond et al. (1966) suggested that the focal deposition of immunoglobulins was secondary to proteinuria and not of primary pathogenic importance.

In 1971 Gerber and Paranetto (1971a) dexcribed the deposition of IgE within mesangial regions in four of five patients with minimal lesion glomerulonephritis. These authors had no explanation for the presence of this immunoglobulin and unfortunately this report has not been confirmed by other authors (Roy, Westberg and Michael 1973). In this series I have examined the biopsies of three patients with specific anti IgE anti-serum and they were all negative. The deposition of fibrin in the glomeruli of patients with minimal lesion glomerulonephritis has been described previously (Jao et al. 1973). In this study a small amount of fibrin/fibrinogen was demonstrated in eight of twenty-four biopsies. The cause for this deposition is not known. It seems unlikely that it is part of an inflammatory reaction in view of the negative findings as far as immunoglobulins and complement are concerned. In addition, on electron microscopy there is little or no evidence of any abnormality in the glomerular capillary lumen or in subendothelial position. It is possible, however, that small amounts of fibrin are deposited as a consequence of the nephrotic syndrome. It is known that in this syndrome there is a hypercoagulable state, and frequently diminished fibrinolysis. This could lead to the deposition and inadequate removal of small amounts of fibrin in capillary lumina and may therefore account for the material seen in this series.

Minimal lesion glomerulonephritis is a difficult condition to diagnose without adequate clinical information and electron microscopic examination. In this study it accounts for only two-thirds of all children presenting with the nephrotic syndrome; this underlines the importance of accuracy of diagnosis. The prognosis in these patients is uniformly good, apart from the fact that they may develop intercurrent infection whilst being treated with steroids or immunosuppressive agents. The long-term prognosis is more difficult to establish, as it is a disease characterised by remissions and relapses. It is more than likely that interesting data will be forthcoming on the long-term (greater than 20 years) follow up of patients who have been properly diagnosed.

8. FOCAL GLOMERULOSCLEROSIS

Focal glomerulosclerosis (focal sclerosing glomerulonephritis) is a condition which is clinically indistinguishable from minimal lesion glomerulonephritis. It usually presents as the nephrotic syndrome and is most common in childhood. Unlike minimal lesion glomerulonephritis, however, there is a slight female preponderance in this condition.

Before this condition was adequately documented it was noted that certain patients who clinically and even histologically appeared to have the feature of minimal lesion glomerulonephritis developed chronic renal failure and the glomeruli at post mortem appeared to be uniformly sclerosed. Certain reports have documented the progression of this condition by serial biopsies, (McGovern 1964, Hayslett et al. 1969 and Heptinstall 1974). In an autopsy study on children Rich (1957) demonstrated that the juxtamedullary glomeruli were the first to be involved in the sclerosing process and this then spread outwards to involve progressively more of the cortex. It is highly likely that this condition is not a variant of minimal lesion glomerulonephritis but a separate entity. Clinically there are certain features which may distinguish it from minimal lesion but these were not seen in the patients studied in this series.

Immunofluorescence examination of this condition shows the most common deposit to be IgM associated with C_3 in glomerular capillary walls (McGovern and Lauer 1973, Hyman and Burkholder 1973). In the present study there was no consistent or characteristic immunofluorescence pattern. However, many cases have been described like two in our series in which there is no immunofluorescence detected.

In the majority of patients there is little or no response to

corticosteroid therapy. Only one of 22 described by White, Glasgow and Mills (1973) showed any response to steroid therapy. In a series reported by Habib and Gubler (1973) 26 of 79 had some response to cortico-steroid therapy but in only one patient was there permanent improvement in renal function. These reports are in contrast to one of the patients in this study who appeared to make a very satisfactory and complete recovery following steroid therapy.

Focal glomerulosclerosis is described as an unusual condition in childhood, in its initial presentation indistinguishable from minimal lesion glomerulonephritis. However, in this series there is only one child in the five patients examined. Indeed one patient (case 89, aged 71) is one of the oldest patients in this series. An interesting feature of the present group is that at initial presentation four had significantly reduced renal function (creatinine clearance less than 40 mls per minute) and hypertension. It is possible that focal glomerulosclerosis is only a varient of diffuse proliferative glomerulonephritis in which the immunological insult or glomerular response is such as to produce a progressive hyalinisation of the glomeruli. There is no known therapy for this condition and like most forms of glomerulonephritis the aetiology is unknown.

9. HENOCH-SCHONLEIN PURPURA

The Henoch-Schonlein syndrome is characterised by a purpuric skin eruption occurring on the extensor surfaces of the arms and legs as well as on the buttocks and lower back, associated with joint pains, abdominal pains and haematuria. These components may not all be present in any given patient. The condition is found most commonly in children, usually males, but it occurs also in adults. It is frequently associated with renal involvement which may, if severe enough, lead to renal failure and death. The purpura and joint pains were initially described by Schonlein in 1837 and Henoch (1874) added the gastro-intestinal symptoms and later (1899) drew attention to the frequent renal involvement.

Renal involvement in Henoch-Schonlein purpura varies from 12% (Roberts et al. 1962) to 63% (Kobayashi et al. 1965) depending upon the patients studied. The present series of eleven patients gives no indication as to the frequency of renal involvement in this syndrome. Although it occurs most commonly in children it has been reported in adults (Cream et al. 1970, Ballard et al. 1970) and in the present series three of the eleven patients were over 17 years old.

The histological appearances in this condition are extremely variable. It may be normal even when urinary abnormalities are detected (Heptinstall 1974, Ballard et al. 1970 and Fillastre et al. 1970). In some patients there appears to be a focal glomerulonephritis in which only some glomeruli are affected and these may show only segmental lesions. Other patients reveal a mild diffuse proliferative glomerulonephritis, whilst others who have rapidly progressive renal failure may show extensive crescent formation and glomerular capillary

thrombosis. In the present series the most common finding was a minor diffuse proliferation of mesangial and endothelial cells, although in three cases the proliferation appeared to have a focal distribution. Four patients in this series showed crescent formation and three of these patients progressed to intermittent haemodialysis or died.

The immunofluorescence findings have been variably reported. In this series the most common finding was of IgG, IgA and fibrin within glomerular capillary walls. This is in contrast to the findings of Fillastre et al. (1970) who found intense diffuse staining of IgA, IgG and B 1C in mesangial areas in adults with Henoch-Schonlein purpura. Berger et al. (1971) found that IgA was invariably the main component, that it stained more strongly than either IgG or C_3 and was present predominately in the mesangium. This was not confirmed in the present study. In addition the finding reported by Urizar et al. (1968) of linear immunofluorescence was not seen in this study. The differences in the immunofluorescence findings between various groups is difficult to explain. In this syndrome it is difficult to mistake the diagnosis because of the characteristic clinical picture. Thus differences in the biopsy findings must be either due to the biopsies being taken at different times in the natural history of the disease or being examined with different antisera. This has already been discussed in relationship to mesangial IgG/IgA disease and there is little doubt that the same factors apply with respect to Henoch-Schonlein disease. This emphasises the fact that when describing the results of immunofluorescence examinations full details of the technical methods must be included.

The prognosis in this condition is variable. To a large extent it depends upon the severity of the renal involvement and the most important prognostic indicators revealed by the present study would seem to be diminished renal function at presentation or the presence of crescents seen by light microscopy. The pattern of immunofluorescence deposition did not appear to bear any relationship either to the initial presentation or to the eventual outcome. The fact that three of the eleven patients in this study progressed to end stage renal failure may seem to give an unduly gloomy prognosis. While this finding compares with that of Ballard et al. (1970) it is probably biased as many patients with Henoch-Schonlein disease are not subjected to renal biopsy. It is interesting to note, however, that of the four patients in the present series who showed crescent formation three progressed to chronic renal failure.

10. ACUTE TUBULAR NECROSIS

There is considerable confusion in the literature regarding the terms acute tubular necrosis and acute renal failure. Acute renal failure must be used to describe a clinical syndrome characterised by the sudden onset of reduced renal function sufficient to result in the accumulation in the blood of those substances normally excreted in the urine. There are many causes of this syndrome, of which acute tubular necrosis is only one. For instance, in this study patients presenting with acute renal failure have been found subsequently to suffer from such conditions as rapidly progressive glomerulonephritis, Goodpasture's Syndrome, hypertension and polyarteritis. In this section the findings in such patients are excluded, and only those with histological evidence of acute tubular necrosis consequent upon some single specific insult will be considered. However, it can be seen that acute tubular necrosis may occur from a wide variety of insults such as sepsis, poisoning, hypotension and multiple myeloma.

The pathogenesis of this condition is unknown, but it is highly likely that there is more than one way in which the lesion may be produced. The tubular cells of the nephron are metabolically highly active, and as such are susceptible to any reduction in the supply of oxygen and other essential metabolites. In addition the blood supply to the proximal tubule is unusual; the arteriole first enters the glomerulus as the afferent arteriole, devides into a capillary plexus and then leaves the glomerulus as the efferent arteriole. This vessel then further divides to form the peritubular capillary plexus. The capillaries supplying the tubular cells are thus unique in that they are the second of two networks between the initial arteriole and

the draining venule. The tubular cell is susceptible to a reduction in perfusion of this second capillary network, and this probably explains why acute renal failure and acute tubular necrosis may be seen in conditions severely affecting the afferent arteriole such as malignant hypertension, scleroderma and polyarteritis nodosa, and conditions affecting the glomerulus such as rapidly progressive glomerulonephritis. In states of shock and hypovolaemia where there is diminished renal perfusion there is renin release and it has been postulated that this acts locally in the kidney causing severe efferent arteriolar constriction in an attempt to maintain glomerular filtration. Such an event, if severe enough and of sufficient duration could cause ischaemia and necrosis of the tubular cells and/or basement membrane. Plasma renin activity is increased in most patients with acute renal failure during the period of renal insufficiency (Kokot and Kuska 1969) and returns to normal as renal function improves. In addition it is likely that the tubular cells could be directly damaged by 'toxic' substances as in septicaemia or from drugs or chemicals.

In this study acute tubular necrosis was associated most commonly with sepsis. Two patients had a chemically induced acute tubular necrosis, one with paracetamol and one with sodium chlorate. It is not known whether sodium chlorate, a weed killer, acts directly upon the proximal tubular cell or whether it causes its injury by massive red cell disruption with subsequent liberation of haemoglobin, producing a lesion similar to that seen in the crush limb syndrome. Multiple myeloma is thought to cause acute tubular necrosis by deposition and condensation of myeloma protein in proximal tubular

lumina. Early reports suggested that intravenous pyelography caused the deposition of myeloma protein by some interaction with the contrast media, but it is now felt that the initiating event is the preceding dehydration which is fairly standard for patients undergoing intravenous pyelography. It is interesting to note that the patient in this study had undergone dehydration twice in the space of a few days, once for surgery and once for pyelography, and that she developed acute tubular necrosis from which no satisfactory recovery occurred.

Recently considerable interest has centered around the role of fibrin deposition in the pathogenesis of acute tubular necrosis. Clarkson et al. (1970) demonstrated fibrin in the glomerular capillary lumina in this condition, in some cases sufficient to cause capillary occlusion. It is not known whether this occurs as a primary event or as a consequence of some other vascular injury. In only four of the eleven biopsies in this study was any fibrin/fibrinogen demonstrated in glomerular capillary walls. This is in contrast to the electron microscopic findings of Clarkson et al. (1970). It is however, possible that positive immunofluorescence was not detected because of the dynamic nature of fibrin. Once fibrin is deposited it is subject to the action of a stabilising factor, Factor XIII, and subsequently to the effect of fibrinolysins. Thus it is subject to a variety of influences which may render detection by immunofluorescence impossible. In addition the amount of material which can be detected by electron microscopy and by immunofluorescence are different and it is possible that the amount of fibrin commonly deposited in acute tubular necrosis is less than that detectable by

immunofluorescence.

In this study the most common finding was a diffuse staining of the oedematous intertubular tissue with fibrin/fibrinogen. This may be only a reflection of the inflammatory exudate which is seen on light microscopy, and this is supported by the detection of interstitial cells containing immunoglobulins which by light microscopy appear to be plasma cells. Similar findings are seen in rejection episodes in transplanted kidneys (see IV 17). The diffuse intertubular fibrin/fibrinogen staining and cellular infiltrate of immunoglobulin containing cells appeared to be the most consistent immunofluorescence finding in acute tubular necrosis, and probably reflects only the inflammatory response which accompanies such an event.

Two biopsies, obtained late in the illness, showed IgM and C_3 in glomerular capillary walls. Similar findings were observed in one patient with acute renal failure due to disseminated intravascular coagulation and in whom serial biopsies were obtained (Case 122, see IV 11). It is possible that in these three patients some antigen had been liberated from the kidney or associated with the initial insult which then led to the deposition of IgM, a first-phase antibody, and complement in the glomerular capillary wall. In two of the patients it is possible that the antigen was introduced as a result of infection (leptospirosis in Case 141, influenza A in Case 122) but in the other patient the acute tubular necrosis developed consequent upon an anti-partum haemorrhage and so it is most likely that a tissue antigen, possibly even basement membrane itself, is liberated in some patients during acute tubular necrosis with subsequent antibody formation.

The biopsy findings gave no indication as to the prognosis of the illness except in the patient with multiple myeloma where the degree of tubular damage was such as to make recovery unlikely. The prognosis appears to depend most upon the cause of the tubular necrosis, with a mortality of 58% in a surgical group and 21% in an obstetric group, and an overall mortality of 44% (Kennedy et al. 1973). Thus in the present group of nine patients the mortality compares favourably with other series.

11. DISSEMINATED INTRAVASCULAR COAGULATION

Disseminated intravascular coagulation (DIC) (diffuse intravascular coagulation, consumption coagulopathy, haemolytic uraemic syndrome, thrombotic thrombocytopaenic purpura) is a recognised syndrome which may be caused by a wide variety of agents and may present in many ways. Localised intravascular coagulation has long been associated with the inflammatory reaction and the presence of fibrin/fibrinogen in the glomeruli of patients with many differing forms of glomerulonephritis supports the fact that localised coagulation is frequently involved in glomerular inflammation. In disseminated intravascular coagulation, however, there is widespread fibrin formation and deposition unrelated to any localised focus of inflammation. The hallmark of DIC is the fact that the formation of fibrin is extensive enough to result in a fall in the plasma fibrinogen concentration and an associated 'consumption' of other coagulation factors producing a subsequent fall in their plasma concentrations. In addition the fibrin deposition results in the trapping of platelets and the disruption of red blood cells, producing thrombocytopenia and haemolytic anaemia. The reduction in coagulation factors and the thrombocytopenia are frequently severe enough to cause bleeding. The clinical presentation will, however, depend upon the extent of consumption of these factors and also on the nature of the agent initiating the coagulation.

There are many pathological states associated with DIC but infections especially seem to play a significant role in contributing to this syndrome. In children there is frequently a prodromal phase of diarrhoea and gastrointestinal upset followed a few days later by an abrupt onset of acute renal failure, anaemia and bleeding disorders

(Gianantonio et al. 1964). Two of the present group presented in this way, of whom one was an adult (case 261) and one a child (case 77); although it is most likely that their symptoms were due to a virus infection a search failed to reveal any pathogenic organisms. Two patients (case 122 and case 124) developed DIC as a complication of influenza A virus infection. Both presented within a week of each other during a minor influenza epidemic and must therefore represent an unusual response to a virus which was epidemic and relatively innocuous to the majority of people it infected. The first case made a satisfactory recovery after a period of acute renal failure while the second died of fulminating DIC associated with a massive haemoptysis. It is interesting to note that the case described by Goodpasture 1919 was of a young man who developed haemoptysis and died during an attack of influenza. It is just possible that this was a case of DIC rather than an antibody-mediated disease. One other patient in this group developed DIC in association with an infection (case 130). This young boy was being treated with steroids for an underlying minimal lesion glomerulonephritis when he developed a pneumococcal meningitis associated with a fatal DIC. Thus of the seven patients in this study five developed DIC in association with a proven or suspected infection. The association between infection and DIC is well documented and these five patients were fairly typical cases (Yoshikawa et al. 1971).

The two remaining cases are rather unusual. The first patient (case 3) developed DIC after a three week course of ethynol oestradiol. It is unlikely that this was the sole agent responsible for the development of DIC as she had a short prodromal history of feeling tired.

The second patient (case 32) developed DIC while being treated with ACTH for Crohn's Disease. In neither of these patients was any infecting agent isolated but it is not possible to state that the DIC was not initiated by an infection. However, it is known that coagulation can be precipitated by treatment with oestrogens (Brown et al. 1973) and also by steroids and so it is possible that these therapeutic agents were responsible in part for the condition in these cases.

All patients except one (case 77) had acute renal failure at the time of initial referral. They could therefore be considered as having the haemolytic uraemic syndrome. There is little point in trying to differentiate between the haemolytic syndrome and DIC, since the only factor which will determine if the 'uraemic' component will develop in any given patient is the extent to which intravascular coagulation affects the functioning of the nephron.

The immunofluorescence findings are as would be expected knowing the pathogenesis of this condition. The most common finding was of fibrin deposition in glomerular capillary walls, often to the extent of occluding the capillary lumina, and also fibrin deposition in small arterioles, again frequently causing occlusion. In three patients a small amount of IgM was present in a granular distribution in glomerular capillary walls; all three patients had underlying infection, and two were the cases of influenza A infection referred to above. It is possible that IgM represents an early phase antibody response to the infection. In two patients C_3 was also detected with the fibrin, one associated with IgM and one with no immunoglobulin deposition. This latter case is of interest in as much as it

illustrates that complement activation may occur in coagulation. It is possible that platelets enmeshed in the fibrin strands release a factor which activates the complement cascade, resulting in the deposition of complement (Kalowski et al. 1975).

Fibrin was detected in the mesangium of only one patient. There are several possible explanations for this lack in the presence of considerable amounts in the capillary lumen. It is possible that there is a time relationship between the deposition of fibrin and its subsequent removal via the mesangial cells. All the patients in this study were biopsied at an early stage in their illness and so it is possible that lysis and/or phagocytosis had not had sufficient time to be effective. However, one patient (Case 122) had three biopsies throughout her illness and in the latter biopsies, although there was significant clearing of the fibrin from the capillary lumina, none was detected in the mesangium. This suggests that the fibrin is lysed in the lumen and then removed by passage of the fragments on to the systemic circulation or into the urine. The latter possibility is supported by the finding of Clarkson et al. (1970) who showed that in the recovery phase of acute tubular necrosis there is a "wash-out" of fibrin degradation products in the urine. An alternative explanation would be that the fibrin or fibrin degradation products contained in the mesangium are in a form which is not suitable for detection by immunofluorescence.

In several patients fibrin was visible as a diffuse staining in the intertubular area rather similar to that seen in acute tubular necrosis. The majority of cases showed evidence of acute tubular necrosis, and this fibrin is probably a manifestation of the

inflammation seen in such cases (see V 10).

The treatment of DIC is controversial. One patient in this group was treated with heparin but unfortunately died from a cerebral vascular accident. Treatment of DIC with heparin has had its advocates (Brain et al. 1968) but several workers have pointed out difficulties in the management of patients with this drug, particularly when it is accompanied by other drugs such as fibrinolytic and/or antiplatelet agents (Clarkson et al. 1969, Powell and Ekert 1974). Three patients in this group were not treated with any anticoagulant and they made a satisfactory recovery. This is similar to a report by Corrigan et al. (1973) who described three paediatric patients with DIC who made satisfactory recoveries without anticoagulant therapy. It is difficult to draw any firm conclusions from these anecdotal studies but it is probably best to reserve heparin therapy for those patients in whom the DIC produces such a degree of coagulation consumption that life-threatening bleeding occurs. In other patients it is highly likely that the DIC has resulted from some single episode and therapy should be aimed at the eradication of the infection and at the consequences of the DIC such as acute tubular necrosis.

In many ways DIC can be considered as a complication of numerous different pathological conditions. The prognosis in such cases will therefore depend upon the nature of the underlying pathology and the degree of consumption of the coagulation factors. Of the 23 patients in this study who presented with acute renal failure seven had DIC, and while this may give a false indication of the incidence of DIC there is little doubt that it is more common than generally supposed and will be detected only by careful investigation and an awareness

of its manifestations.

12. GOODPASTURE'S SYNDROME

The association of glomerulonephritis and haemorrhagic pneumonitis was first described by Goodpasture in 1919. Since then there have been many reports of haemoptysis associated with severe glomerular proliferative and necrotising changes (Parkin et al 1955, Stanton and Tange 1958 and Prosky et al. 1970).

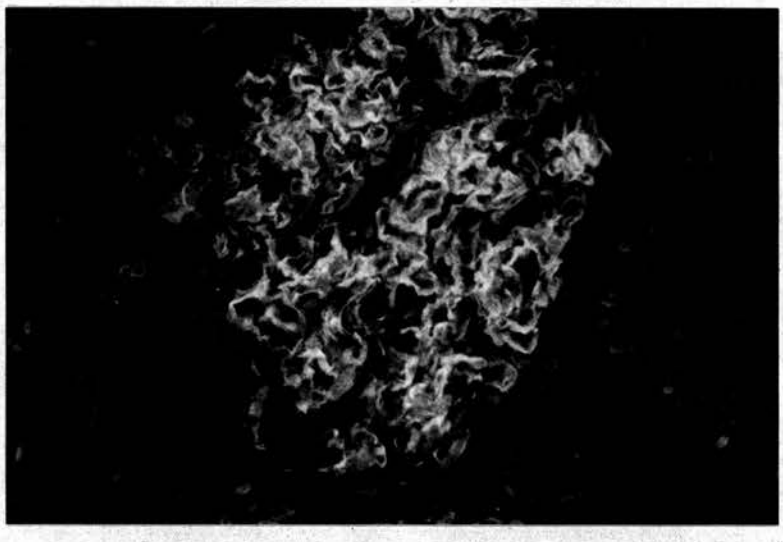
The majority of cases occur in the third decade, and there is a marked male predominance. The presenting symptom may be haemoptysis or haematuria, and on clinical examination the patient is usually normotensive. Sometimes, as in the patient in this series, the clinical presentation is that of acute renal failure.

Histologically the lesion may be a focal glomerulonephritis in the early stages but this rapidly progresses to a stage where there is widespread, large epithelial crescent formation, i.e. rapidly progressive or crescentic glomerulonephritis (Benoit et al. 1964, Johnson and McGovern 1962). In the majority of patients there is linear immunofluorescence to IgG and C_3 along glomerular capillary walls. In some cases there is staining only for IgG but not for C_3. In the present case linear IgG deposition was present in association with small amounts of complement (C_3). There was also fibrin deposition in the numerous circumferential crescents, similar to that reported by Duncan et al. (1965) and Koffler et al. (1969). The prognosis in this condition is extremely poor. There have been several case reports of remission but the vast majority of patients die within a few months of the renal disease becoming manifest. It is interesting to note that this patient had no further haemoptysis after bilateral nephrectomy. This has been reported by other workers

(Maddock et al. 1967 and Nowakowski et al. 1971); there is no explanation for this interesting phenomenon except that the nephrectomy may be removing the source of the stimulating antigen.

This condition is considered to be mediated by an antibody against the glomerular basement membrane. Diagnosis depends on the demonstration of circulating antibody (Fig. 100). Although in some cases the production of this antibody has occurred following an upper respiratory tract infection, the vast majority arise spontaneously in the absence of any associated respiratory disease. The case reported in this study had linear deposition of immunoglobulin along the basement membrane, confirming the concept of an anti-GBM mediated condition.

Figure 100



Linear deposition of IgG on the basement membrane of a normal glomerulus which had previously been covered with serum from a patient with Goodpasture's syndrome demonstrating the presence of anti-glomerular basement membrane antibody.

13. SYSTEMIC LUPUS ERYTHEMATOSUS

The diagnosis of systemic lupus erythematosus is often difficult. However, following the recommendations of the American Rheumatism Association it is now generally accepted that if four of fourteen different clinical criteria are present, a diagnosis of S.L.E. can be made (Cohen and Canoso 1972). In the present study all ten patients had at least four of the fourteen criteria. It is predominantly a disease of females and this is borne out by the fact that all ten patients in the present series are female. In addition, as its name suggests it is a systemic disease with involvement of many systems, which accounts for the diverse ways in which the disease may present. It tends to affect younger people, and the mean age onset of symptoms in the present series of 27.8 years accords well with that described by Estes and Christian (1971) who found a mean age of onset of 30 years in their series of 150 patients.

Renal involvement in S.L.E. is important. It occurs in from 37 to 70% depending upon which series is studied. The reason for the importance is the fact that it is a frequent cause of death in all reported series. Renal involvement may be detected either because of routine urine analysis in patients known to have lupus erythematosus or because the renal manifestation is the first indication of a systemic disease. Occasionally as in case 180 in this series, the patient present with acute renal failure and in such cases a prognosis is extremely poor despite the use of high doses of corticosteroids and immunosuppressive drugs (Baldwin et al. 1970, Cameron et al. 1970, Zech et al. 1972), although Ponticelli et al. (1974) had described the recovery of function in three of four such patients treated with

large doses of Prednisone and heparin. The one patient with acute renal failure in this series did not respond to high dose steroid therapy.

There is no characteristic histological appearance. The findings may vary from normal to a diffuse or focal proliferative glomerulonephritis with or without foci of necrosis. Some patients exhibit a variable degree of capillary wall thickening which has been termed the 'wire loop' lesion. In other patients the appearances are those of a membranous glomerulonephritis of variable extent. In this study some three patients were found to have focal proliferative lesions and seven showed diffuse proliferation. The histological appearance appears to be of some prognostic significance.

Focal glomerulonephritis occurs in about one-third of patients with lupus erythematosus (Baldwin et al. 1970, Estes and Christian 1971, and Pollak et al. 1964), and this accords well with the findings in the present series. Although it has been stated that proteinuria is usually mild in such patients, one of our patients had a protein excretion of 10 grams per 24 hours. The prognosis in patients with S.L.E. who have a focal lesion appears to be good (Baldwin et al. 1970, Striker et al. 1973) although Zimmerman et al 1975 found no difference in the survival statistics of patients with focal or diffuse lesions, and indeed found four patients who presented initially with focal lesions and progressed to diffuse changes. In our series, in which only three patients exhibited focal lesions, it is impossible to draw any conclusion regarding prognosis, but one of these patients showed definite deterioration in renal function and eventually died from chronic renal failure.

The most common and serious form of lupus nephritis appears to be the diffuse proliferative variety. It occurs in approximately 50% of patients with S.L.E. and was present in seven of the ten patients in this study. In most reported series the prognosis in such patients is poor, and this is borne out by the fact that in our patients five showed serious deterioration in renal function or died from chronic renal failure, whilst one remained fairly static and only one showed any evidence of improvement in renal function.

The immunofluorescence findings in the present study agree well with that reported in most other series. In the focal proliferative lesion there may be either diffuse or patchy distribution of IgG in the mesangium, possibly associated with IgA, IgM and C_3 deposition. This appearance was present in one of the three patients in this series. In addition to mesangial deposition, IgG may be present in the capillary walls, even in glomeruli apparently unaffected by light microscopy. Such was the case in two of our three biopsies. In patients with a diffuse proliferative lesion immunoglobulins and complement were commonly deposited in glomerular capillary walls and present only rarely in mesangial regions. This is certainly supported by the present series where there was mesangial deposition in only two of the seven patients. Out most common finding was IgG deposition in glomerular capillary walls associated with fibrin/fibrinogen deposits. IgM was rarely present and IgA was detected in only one patient. Nuclear localisation of immunoglobulins has been described in renal biopsy specimens (McCoy 1972) but in the present series this was only marked in one case (see Fig. 76).

From the present study it is not possible to draw any conclusions

regarding those parameters which seemed to be of some prognostic significance. There does not appear to be any relationship between the age at which symptoms become manifest, the length of the symptomatic history, the presence of haematuria, degree of proteinuria or presence of hypertension and the outcome. However, two factors appear to have some importance. There is little doubt that as in other reported series patients with diffuse proliferative lesions have a worse prognosis than patients with focal proliferative lesions. In addition, the development of acute renal failure in patients known to have lupus erythematosus appears to carry a poor prognosis.

The present study gives little help in elucidating the pathogenesis of this condition. No patient had been taking any drug known to precipitate S.L.E. nor did there appear to be any common factor in the past history of these patients.

14. POLYARTERITIS NODOSA

Polyarteritis nodosa was initially called periarteritis nodosa and described as a condition in which there were nodular swellings along the course of medium sized muscular arteries. Microscopically there is an infiltration of inflammatory cells in the outer part of the vessel walls, together with fibrinoid degeneration. Ferrari (1903) suggested the term polyarteritis because the changes occurred within the vessel wall itself and not outside it. Recently it has become recognised that similar changes may occur in vessels of all sizes including capillaries and venules. The term polyarteritis therefore should not be confined to conditions affecting only medium sized arteries.

Currently it is considered that there are two main types of polyarteritis, classical form and a microscopic form (Davson et al. 1948). In the classical form there is involvement of medium sized muscular arteries, whilst in the microscopic form the main changes occur in small vessels and capillaries. In this study all patients have had the microscopic form of polyarteritis and no cases of the classical type have been seen. Renal involvement in polyarteritis nodosa is common, 70% to 82% in various published series (Patalano and Sommers 1961, and Griffith and Vural 1951). From the present study it is not possible to give any indication as to the incidence of renal involvement in polyarteritis nodosa as all patients studied had renal involvement as an indication for biopsy. However, the present study confirms the fact that it is more common in older patients and that there is some male preponderance.

The clinical features of this disease will depend to a large

extent on the degree of vascular involvement in various organs. The clinical manifestations are protean and this is due to the fact that vessels may be involved in any part of the body. However, in most series pulmonary involvement appears to be relatively common and this is borne out by the fact that in half the patients studied in the present series the initial presentation was with respiratory symptoms. However, arthritis, polyneuritis, cardiac failure and renal failure are all well recognised modes of presentation.

Hypertension seemed to be uncommon in the present group and this is contrary to several other reports (Harris et al. 1939, Rawlson and Kvale 1949, and Rose and Spencer 1957). It has been stated that hypertension develops after renal involvement (Rose and Spencer 1957) but this would not be supported from the present study, as all patients biopsied had evidence of renal involvement. It has also been stated that the classical type in which medium sized muscular arteries are involved is more commonly associated with hypertension, but as no patients with this particular form of disease have been seen no comment can be made on this. The degree of renal involvement in the present series has been extremely variable and it is difficult therefore to explain the absence of significant systemic hypertension.

There are few reports on the immunofluorescence findings in this condition. Paronetto and Strauss (1962) described the deposition of immunoglobulins and fibrin in the glomeruli. Berger and his co-workers (1971) described the deposition of fibrin in the glomerular lesions but he had studied only three patients. Freidman and his co-workers (1960) described immunoglobulins deposited in glomerular capillary walls. In the present group of patients there is considerable

immunoglobulin deposition, with some complement and fibrin. In only two cases was any immunofluorescence detected in arterioles. In one there was deposition of C_3, whilst in the other there was IgM. The most common finding was of a small amount of fibrin deposited in glomerular capillary walls.

The aetiology of this condition is unknown. However, experimentally it has been shown that the injection of foreign protein into the rabbit can produce lesions essentially similar to polyarteritis nodosa (Germuth 1953). In this experimental model it is considered that immune complexes are formed and that these are responsible for the arteritic lesions. In the present study the finding of immunoglobulins, complement and fibrin in a considerable number of the patients would support an immune complex mediated disease. This is further supported by the findings of Clark and Kaplan (1937) in which lesions similar to polyarteritis nodosa were seen in patients dying during serum sickness, and by the report of Fordham et al. (1964) in which similar lesions were seen in three patients with poststreptococcal glomerulonephritis. More recently the hepatitis B antigen (Australia antigen) has been implicated and has been demonstrated in the blood vessel walls and in circulating immune complexes in one patient (Gocke et al. 1970). It seems highly likely therefore that polyarteritis represents an unusual form of an immune complex mediated disease.

Wegener's syndrome is a variant of polyarteritis nodosa in which there is upper respiratory tract granuloma associated with fibrinoid necrosis of arterioles and/or focal areas of necrosis in glomeruli (Wegener 1939). Frequently, as in the patient studied in the present series, the initial presentation is due to nasal involvement

(Walton 1958). Renal involvement is extremely common and approximately half of the patients with this syndrome die from renal failure.

The microscopic and immunofluorescence findings in this condition are in no way different from microscopic polyarteritis, and this was certainly true of the patients studied in this series.

The prognosis of this condition has altered considerably since the introduction of immunosuppressive therapy. The present patient is maintained in a fairly stable state on steroids and azathioprine.

The aetiology of this condition is unknown and unfortunately the present case provides no useful pointers.

15. SCLERODERMA

Scleroderma (Progressive Systemic Sclerosis) is an unusual condition in which there is widespread non-specific fibrinous intimal thickening of arteries and arterioles narrowing the vascular lumina. This condition may affect blood vessels in the skin, gastro-intestinal tract or kidney. It is commonly associated with Raynaud's phenomenon, a positive rheumatoid factor, antinuclear factor and other auto-antibodies. The incidence of renal involvement is unknown. Tuffanelli and Winkleman (1961) investigated 727 patients with scleroderma and found proteinuria in 109. However, serious renal disease was present in only 19 of these patients, and was the cause of death in only 15. This is in contrast to the findings of Cannon et al. (1974) who found that 45% of 210 patients with scleroderma had clinical evidence of renal involvement. The present study contains only two patients with scleroderma and therefore it is impossible to draw any conclusions regarding either the incidence of this condition or of the frequency of renal involvement.

The presence of renal involvement in patients with scleroderma is associated with a poor prognosis. A study of Medsger and Masi (1973) showed that all their patients with clinically evident renal disease died in less than twelve months. In addition Cannon et al. (1974) showed that only 10% of 116 patients without renal disease died during their study whereas 60% of 94 patients with renal involvement died in the same period. As well as carrying a poor prognosis renal failure appears to be a common cause of death in patients with scleroderma. Renal failure was the cause of death in 43% of patients reported by Cannon et al. (1974), 48% of those reported

by Heptinstall (1974c) and 51% of Rodnan (1963). Thus renal involvement is not only common in scleroderma but it is associated with a poor prognosis.

Both patients in this study revealed deposition of IgM in the thickened intima of arterioles. This is similar to the findings of McGiven et al. (1971) who found that IgM was the predominant immunoglobulin in arterioles and small arteries. Fennel et al. (1961) were able to demonstrate only small amounts of immunoglobulins within arterioles, whereas fibrinogen was frequently detected.

The aetiology of scleroderma is unknown but it appears to bear some relationship to the so-called connective tissue diseases. The finding of IgM within arterial and arteriolar walls, however, does not necessarily indicate that it is of prime importance in the pathogenesis of the condition since immunoglobulins can be detected within the thickened intima of arterioles in malignant hypertension. It is possible that scleroderma represents a form of intravascular coagulation, as some cases exhibit some of the clinical abnormalities of microangiopathic haemolytic anaemia (Salyer et al. 1973).

16. HYPERTENSION

Hypertension is frequently associated with renal disease. It can occur in association with most forms of glomerulonephritis and in many conditions that affect the kidney as part of a systemic disease, such as systemic lupus erythematosus, polyarteritis and amyloidosis. Hypertension may occur also as a primary event and cause secondary damage within the kidneys. It is sometimes difficult in the later stages of hypertension to determine whether there has been any underlying renal cause for the raised pressure or whether the renal abnormalities are a direct consequence of hypertensive vascular disease.

In the present study some 21 patients with hypertension were considered to have no obvious underlying renal cause for their disease. Immunofluorescence examination of eleven of these patients who had essential hypertension was negative. The remaining ten patients exhibited malignant hypertension and of these five had significant fibrin deposition in glomerular capillary walls. There have been few other reports of the immunofluorescence findings in malignant hypertension. Paronetto (1965) stated that fibrinogen was invariably present in necrotic arterioles and in the glomeruli which exhibited necrosis. The fibrin appeared diffuse along capillary walls and was also within mesangial regions, sometimes associated with IgG and complement in arterioles. Burkholder (1965) described the deposition of fibrin in arterioles and small arteries, but only patchily in a few glomeruli. In his study immunoglobulins were also demonstrated within the intima and media of small blood vessels. It has been suggested that these two studies support an immunological basis for the

hypertension. However, it is just as possible that the immunoglobulins and fibrin were deposited in a non-specific manner due to the intimal damage induced by the raised blood pressure. It is also possible that the hypertension, particularly in a malignant phase, damages endothelial cells, with consequent mural deposition of fibrin and incorporation of other plasma proteins. This may even be severe enough to give some of the clinical indicators of a disseminated intra-vascular coagulation. It is possible that such an event takes place as a consequence of a very high blood pressure and is therefore in some way responsible for the histological appearances of malignant hypertension. On the other hand it is possible that essential hypertension is complicated by local intra-renal DIC, therefore reducing the capillary lumina with consequent reduction in renal perfusion and stimulation of the renin antiotensin system; this would constitute a primary event in the initiation of malignant hypertension. This study does not indicate which of these two hypothesis is correct, but it is interesting to note that in those patients with essential hypertension no evidence of fibrin deposition has been detected. This would seem to indicate that the finding of fibrin within glomeruli and arterioles is a secondary event in patients with malignant hypertension.

17. TRANSPLANTATION

Renal biopsy is important in the management of patients after transplantation. It was suggested by Kincaid-Smith et al. (1968) that a one hour post-transplant biopsy could give predictive information regarding the success of the transplant; if, on average, four or more polymorphs were present in each glomerulus, the outcome was poor. This was not supported by the findings of Perloff et al. (1973) but there is little doubt that an early biopsy is a useful reference with which to compare future biopsies. Following renal transplantation many factors can affect graft function and biopsy is one important way of investigating such events.

Rejection can occur as a hyperacute episode which becomes manifest within minutes of restoring circulation. Thereafter rejection may occur as an acute event any time after ten to fourteen days postoperatively, or occur as a chronic event slowly over the ensuing months. In addition to these modes of rejection a transplanted kidney is liable to develop acute tubular necrosis in the immediate postoperative period and it may also be subject to glomerulonephritis if this was the cause of the original chronic renal failure. The biopsy appearance will therefore depend to a large extent on which of these mechanisms is predominant although it will be clear that more than one of them may be present at any one time.

In the present study ten biopsies were obtained from eight patients in the immediate post transplant period. There have been few previous studies of the immunofluorescence appearances at this time in transplanted kidneys. The most common immunofluorescence

finding was of a diffuse scattering of cells containing IgM within the interstitial regions. There was also a diffuse interstitial staining with fibrin. These findings are very similar to that of acute tubular necrosis and it is known that such an event frequently follows cadaver transplantation, and usually recovers within two to four weeks. Such was the outcome in the majority of patients in this study. Horowitz et al. (1965) found that there was infiltration of IgG containing cells in the interstitium of dog kidneys fifteen days after transplantation. In acute rejection there are also reports of IgG or IgM containing cells in the interstitium, but it is difficult to know whether this represents part of the rejection episode or an event associated with the frequently accompanying acute tubular necrosis.

The immunofluorescence microscopy of rejection episodes showed two major findings. The most common was the appearance of IgM, complement and fibrin/fibrinogen in the walls of small arterioles. This is similar to the finding of McKenzie and Whittingham (1968) who found that IgM was the most common immunoglobulin to be found in arterioles during rejection episodes. He found in addition significant quantities of IgG, complement and fibrin, whilst it was rare to find any material in the blood vessels of non-rejecting kidneys. Pasternak and Linder (1971) found IgA to be present in the blood vessels of six of thirteen biopsies, and IgG and IgM were present in only four instances. The finding of IgA as the predominant immunoglobulin is in contra-distinction to the present study, where it was never detected, and the study of McKenzie and Whittingham (1968) where it was present in only one of 82 biopsies.

Glomerular immunofluorescence was rare in the present study and was detected only to fibrin/fibrinogen. This compares with the study by Porter et al. (1968) who detected linear and granular IgG, complement and fibrin in kidneys 18 days to 8 years after transplantation whether rejection was present or not. Similar findings were reported by Hulme et al. (1972) who found that IgM was deposited in the glomerular basement membrane in 24 of 45 cases. This was associated with IgG in eight, complement in nineteen and fibrin in six. There was no apparent relationship to ultra-structural findings on electron microscopy or to renal function in these patients. The lack of glomerular immunofluorescence in the present study is difficult to explain. In only one patient was there significant deposition of immunoglobulins, complement and fibrin, and it may well be that this represents the only patient who manifested significant humoral rejection. It could be that the other patients represented predominantly cell-mediated rejection and it is known that in such instances the immunofluorescence findings are usually negative.

Two biopsies in the present series are of particular interest. One was obtained two days after transplantation and showed an infiltration of IgM containing cells in the interstitium. This illustrates the rapidity with which a cell mediated response may take place after transplantation. The second case of particular interest is one in which tissue was obtained seven years after transplantation. This showed no evidence of immunofluorescence, a finding similar to one case reported by Busch et al. (1971) who reported negative immunofluorescence in the transplant of a patient surviving for this length of time.

It is difficult to draw any conclusions from this limited study. Certainly in the immediate post transplant period the immunofluorescence findings are more those of acute tubular necrosis. At a later time the predominant lesion appears to be in blood vessels, which is entirely consistent with current knowledge regarding the chronic rejection process.

18. DIABETIC GLOMERULOSCLEROSIS

Renal disease appears to be more common in patients with diabetes mellitus since the introduction of insulin treatment. The reason for this is not entirely clear but it is likely that the increased lifespan gives time for the development of glomerular changes. The pathogenesis of the characteristic lesions of the diabetic kidney, the diffuse and nodular forms of glomerulosclerosis and the exudative lesions, is still unknown. It is possible that diabetic glomerulosclerosis is in some way related to insulin therapy but this seems unlikely as both diffuse and nodular forms may occur in patients treated with either insulin or oral hypoglycaemic agents.

In this study of thirteen patients eight exhibited a diffuse glomerulosclerosis, whilst in three the changes included nodular lesions. The histology in the two remaining patients was normal and they were both in a pre-diabetic phase. There was a paucity of immunofluorescence in these thirteen cases. This was not because they were in an early stage of the disease, since one had been on insulin for at least 24 years. The most common finding was of a weak deposition of fibrin in glomerular capillary walls, and less frequently in mesangial regions. This contrasts with the findings of Thomsen (1972) who found IgG in both diffuse and nodular diabetic glomerulosclerosis. In his study fibrinogen was found only in diffuse lesions and hyalinised arterioles. Similarly the findings of the present study are at variance with those of Westberg and Michael (1972) who found that in twenty of the thirty-seven cases there was linear staining for IgG along the glomerular basement membrane. A similar linear staining was found also in some cases for IgM, albumin, fibrin

and occasionally complement. Farquhar et al. (1972) described the deposition of fibrin in glomerular capillary walls and mesangial regions in fourteen of sixteen biopsies. This finding differs from the present series only in that most of their fibrin appeared to be deposited within mesangial regions, a lesser amount being present in capillary walls.

The differences in the immunofluorescence findings is difficult to explain. It is possible that it represents a difference in the fluorescein conjugated antisera used in each study. Alternatively there may be considerable differences between the populations studied, for instance with respect to the age and sex distribution, duration of diabetes, type of insulin employed and degree of diabetic control.

The present study does not support any immunological cause for the development of diabetic glomerulosclerosis. It is possible that insulin could be contaminated with pancreatic basement membrane and give rise to anti-glomerular basement membrane antibodies. This would support the presence of a linear deposition of IgG in diabetic glomerulosclerosis as found by Westberg and Michael (1972). However, they found little complement deposited on the basement membrane, and eluted IgG from the isolated glomeruli did not react with normal glomeruli in indirect immunofluorescence. These findings, therefore, suggest that anti-GBM antibodies do not play a significant part in the pathogenesis of this condition. Similarly there is no evidence that insulin itself has reacted in an antigenic manner forming antigen/antibody complexes which have become subsequently deposited in the glomerulus. The histological appearances and the absence of subepithelial and/or subendothelial deposits would not support this

concept.

It is possible, however, that the increased permeability of the microvasculature in diabetic subjects is of importance in "trapping" material in a subendothelial position. This may account for the detection of such material as albumin and ceruloplasmin by Westberg and Michael (1972). Their findings could therefore be explained on the basis of increased vascular permeability and subsequent local accumulation of material; they do not give details regarding the diabetic status of their patients, which makes this diffisult to assess. The findings of Mogensen (1976) are of importance in as much as he has demonstrated that the degree of proteinuria and therefore glomerular permeability varies considerably with diabetic control, being much less in those with good control.

The finding of fibrin in the glomerular capillary walls could be explained in a completely different way. It is possible that the diabetic glomerulosclerosis is complicated by proteinuria and therefore a mild degree of hypoproteinaemia. This would result in increased plasma fibrinogen and an increased tendency towards coagulation. In addition to this, some patients with diabetes mellitus appear to have an impaired fibrinolytic response to stress (Cash and McGill 1969) and thus the fibrin deposition and subsequent trapping of other material may be only a reflection of a disordered coagulation mechanism. This is supported by the finding of increased fibrin degradation production excretion in some patients with diabetes mellitus. This disorder may be further compounded by the suggestion that a mesangial lesion is primary in diabetic glomerulopathy (Kimmelstiel et al. 1966), resulting in an impaired ability to clear

accumulating material from a subendothelial position.

This study gives no support to the idea that diabetic glomerulosclerosis is an immunologically mediated injury. It seems more likely that it is a mild coagulation disorder producing an accumulation of fibrin and other materials in the glomerular capillary wall, possibly associated with a disorder of mesangial cell function.

19. AMYLOIDOSIS

The term amyloid refers to a proteinous substance deposited extracellularly in tissues and commonly identified by its characteristic staining with Congo Red dye (Bennhold 1922, Puchtler et al. 1962). These deposits may be restricted to a single organ or be distributed throughout the body. This latter form is known as systemic amyloidosis and may occur as a primary disease or as a secondary condition consequent upon long standing suppurative or granulomatous infections, rheumatoid arthritis, Hodgkin's disease or solid tumours. The characteristic ultrastructurae of amyloid deposits consists of non-branching fibrils of indefinite length but approximately 80 to 100 Å in width (Cohen and Calkins 1959, Spiro 1959 and Shirahama and Cohen 1967). These fibrils are the component responsible for the positive Congo Red staining by which amyloid deposits are identified histologically. By X-ray crystallographic and infra-red analysis this material has been shown to be composed of a protein consisting of polypeptide chains arranged in a beta pleated sheet configuration (Eanes and Glenner 1968, Termine et al 1972).

The pathogenesis of amyloidosis is at present entirely speculative. There are two major chemical classes of human systemic amyloid. The first is of immunoglobulin origin and is usually found in cases associated with multiple myeloma or with plasma cell dyscrasias; in these cases the sites of synthesis of the fibril protein is probably within the monoclonal plasma cells in bone marrow. The second major chemical class of human systemic amyloid consists of fibrils composed of a major protein of presently unknown cellular origin and a lesser quantity of light chain protein predominantly of lambda type. This

second form is the protein usually found in amyloidosis associated with chronic diseases such as rheumatoid arthritis and long standing infection. The ultimate deposition of these proteins and their organ distribution depends upon the rate of synthesis and catabolism, the form of the precursar and its consequent chemical composition, and the tissue affinity for the precursar and its consequent fibril. Amyloidosis occurs in a large number of conditions and there has been little advance in the understanding of this disease over the past 100 years; we may therefore think of amyloid as a 19th century term in search of a 20th century definition (Glenner and Page 1976).

The incidence of amyloidosis is unknown. In this series there are six patients, giving an incidence of approximately 2%. This compares with a recorded 3% incidence in a series of 1500 cases (Triger and Joekes 1973). It is likely that the incidence of secondary amyloidosis is falling, in view of the fact that many of the associated infections are being adequately treated by chemotherapy. In this study three patients appeared to have primary amyloidosis, while three others had a secondary form. The frequency of renal involvement appears to be more common in the secondary form (Auerbach and Stemmerman 1944) than in the primary form, (Symmers 1956).

Proteinuria is an extremely common finding in renal amyloidosis. It is frequently severe and this is born out by the fact that in this study two of the eight patients were excreting amounts in excess of 10 g of protein daily. Renal failure is also common and of the six patients in this study three have died and one progressed to intermittent haemodialysis. Two of the patients died from renal failure. None of the patients in this study had hypertension and this has been noted

previously in patients with amyloidosis, (Brandt, Cathcart and Cohen 1968, Rosenblatt 1933).

The immunofluorescence microscopy findings in this study were disappointing. In one case was there bright deposition of IgG within the homogeneous material which appeared to be deposited in glomerular capillary walls, mesangial regions and arteriolar walls. In a further three patients there was a very weak deposition of fibrinogen in glomerular capillary walls and in one other patient there was a small amount of IgG in glomerular capillary walls. Lachmann et al. (1962) described immunofluorescence to IgG and C_3 in amyloid from various human tissues and similar findings have been reported by Vazquez and Dixon (1956). Bergstrand et al. 1971 described the deposition of IgG and complement ($B^1{}_c$) in seven of nine cases of amyloidosis. The distribution was large masses localised to mesangial areas and capillary walls which they considered distinctive of this condition. There did not appear to be any difference in cases with primary as opposed to secondary forms of this disease. Hietinstall (1974d) describes immunofluorescence to gammaglobulin in glomerular lesions in both primary and secondary amyloidosis, although he does not give the immunoglobulin type or the incidence of this finding. This relative lack of immunofluorescence may be due to the fact that immunoglobulins have only occasionally been found to be present in amyloid deposits (Mellors and Ortega 1956, Vazquez and Dixon 1956). This may be due in part to the restricted antigenic region in amyloid fibrils and to steric factors related to the packaging of the proteins as fibrils (Glenner and Page 1976) which may make the antigenic determinants inaccessible to antibody directed against this portion of

the light chain.

20. MALIGNANCY ASSOCIATED NEPHROTIC SYNDROME

The association between nephrotic syndrome and malignant disease is now well established. In 1969 Cantrel described a case of nephrotic syndrome appearing in a patient who had a gastric carcinoma. The nephrotic syndrome resolved on removal of the underlying tumour by surgery. Squamous carcinoma of the bronchus has also been associated with the nephrotic syndrome (Lewis, Loughridge and Phillips 1971, Loughridge and Lewis 1971) and an oat cell carcinoma of the lung has similarly been described (Higgins et al. 1974). In addition to epithelial solid tumours, there is an association between several types of lymphoma and the nephrotic syndrome. Froom et al. (1972) described a patient with Hodgkin's disease and proteinuria. In 1973 Hyman described a further patient with Hodgkin's disease and also one with Birkett's lymphoma. On reviewing the literature he was able to uncover twenty-one patients with Hodgkin's disease, two with reticulum cell sarcoma and one with lymphosarcoma who had associated proteinuria. Cathan et al. 1974 described two patients with chronic lymphocytic leukaemia and the nephrotic syndrome.

The histological appearances in malignancy associated nephrotic syndrome are variable but the most commonly reported lesion is a membranous glomerulonephritis (Cantrel et al. 1969, Higgins et al. 1974). However mesangiocapillary glomerulonephritis has been reported associated with carcinoma of the breast (Lewis et al. 1971), lymphoma (Muggia and Ultman 1971, Hyman et al. 1973a) and bronchial carcinoma (Heaton et al. 1975). The patients described by Dathan et al. (1974) exhibited a prominent infiltration of a morphous material in the mesangium and basement membrane of all glomeruli, with a consequent

reduction in capillary lumena. This appearance was very similar to that present in the patient described in this series. On immunofluorescence examination IgG, IgM, complement and fibrin has been detected in the glomeruli of patients with malignancy associated nephrotic syndrome.

The pathogenesis of the glomerular deposits is uncertain. It is known that there is a close association between viral infections and lymphomas. It is possible that the virus which initiates a leukaemic reaction is also capable of forming immune complexes, so becoming deposited in the glomeruli. This is supported by the fact that mice infected with oncogenic viruses develop an immune complex type glomerulonephritis, and viral antigen and antibody may be demonstrated in the glomeruli of such animals by an immunofluorescence technique. Higgins et al. (1974) suggest that in the case of solid tumours there may be necrosis of the centre of the tumour with subsequent liberation of nuclear material. There would then be stimulation of antibody formation, subsequent antigen antibody complexes being deposited in the glomerular capillary walls. This was supported by the finding of antinuclear antibodies in the serum of the patient he describes foci of necrosis within the tumour containing large amounts of DNA in an extra-cellular location. As far as the patient described in this series is concerned, the antinuclear factor was negative, which would not support his hypothesis. It is also possible, however, that the tumour itself liberates antigenic material which excites the production of a specific antibody in the host. Loughridge and Lewis (1971) showed that IgG and IgM eluted from the glomeruli of their patient reacted with the surface membranes of bronchogenic tumour cells. In the

patient described in this series it was not possible to perform such an investigation but it is highly likely that a similar reaction was present.

The results of the therapy on the proteinuria and glomerulonephritis is of great interest. Cantrel (1969) described a remission of the nephrotic syndrome and a resolution of the proteinuria following the removal of the underlying gastric neoplasm. Similarly Dathan 1974 describes a considerable reduction in proteinuria in his patient with chronic lymphocytic leukaemia when adequate therapy produced a satisfactory remission of the leukaemia. In the patient described in this series there was a complete resolution of histological appearances with a return of renal function to normal on excision of the choriocarcinoma. It would seem, therefore, that in malignancy-associated nephrotic syndrome it is the continued production of antigen and presumably of antigen-antibody complexes that is important in maintaining proteinuria. Once the source of antigen can be eliminated by either therapy or surgery immune complex formation will be diminished and there will be subsequent resolution of the glomerular lesion.

21. MISCELLANEOUS

Two patients in this study were considered to have renal disease caused by a primary lesion in the heart. The first (Case 223) was found to have subacute bacterial endocarditis on the basis of changing heart murmurs, persistently elevated ESR and blood cultures positive for Strep. viridans. Lohlein (1910) introduced the term 'embolic nonsuppurative focal nephritis' to describe the renal lesion found in some patients with subacute bacterial endocarditis (S.B.E.). He described eight cases in which the causative organism was Strep. viridans, and although this was the organism involved in the present case it is now known that a variety of other organisms can produce the same disease syndrome.

Renal biopsy in the present patient showed typical light microscopy appearances associated with S.B.E. There was focal glomerular hyalinisation, with patches of moderate interstitial fibrosis and focal round cell infiltration. Immunofluorescence microscopy was disappointing in as much as no specific immunoglobulin, complement or fibrin deposition was detected. However there are only sparse reports in the literature on the immunofluorescence findings in patients with S.B.E. Morel-Maroger et al. (1972) described a diffuse granular deposition of C_3 in glomerular capillary walls associated with some IgG and IgM deposition. It is interesting to note that the immunofluorescence was of a diffuse distribution, whereas the light microscopy give the appearance of a focal lesion. This, of course, is the same as the findings in focal proliferative glomerulonephritis (see V 5).

Initially the condition was considered to be of an embolic nature,

small fragments becoming detached from the affected valves and subsequently lodged in the peripheral circulation. It is possible that in some cases this mechanism produces the focal lesion, but most workers now feel that the lesions are produced by the localisation of antigén-antibody complexes in the glomerular capillary walls. This is supported by the finding of a diffuse deposition of complement and immunoglobulins in capillary walls and also by the interesting case of Bain and his co-workers (1958) who described renal lesions in a patient who had endocarditis involving the tricuspid valve. It is, however, unclear as to why the light microscopy lesion should be focal but presumably mechanisms similar to those suggested in focal proliferative glomerulonephritis are active. Unfortunately in view of the negative immunofluorescence findings the present case gives no help in elucidating this problem further.

The second patient in whom the renal lesion was secondary to heart disease was a patient who had undergone aortic valve replacement and who subsequently developed mild proteinuria. Although he had no haematuria it was considered possible that he had an endocarditis with renal involvement. Renal biopsy, however, revealed a considerable deposition of haemosiderin predominately in tubular cells. There was no evidence of any glomerular disease. On further investigation he was shown to have a mild haemolytic anaemia and it was postulated that his homograft valve was producing a constant, but low-grade, red cell haemolysis and that this was leading to haemosiderosis. It is possible that the mild proteinuria was caused by the tubular haemosiderin deposition interfering with the normal tubular reabsorption of the small amount of protein which normally gains access to the proximal

tubule.

One patient with toxaemia of pregnancy has been studied. Toxaemia is a condition of hypertension, proteinuria and oedema which develops towards the end of pregnancy in people who have previously been healthy. It is a rather difficult condition to diagnose, as patients during pregnancy may develop essential hypertension which can be associated with proteinuria, or they may develop glomerulonephritis during pregnancy and this may be associated with hypertension and oedema. In spite of this difficulty it is recognised as a clinical entity which is more common in primigravid patients and has certain characteristic renal biopsy appearances (McCartney 1964).

In toxaemia of pregnancy the glomeruli frequently appear bloodless, and the capillary lumen appears to be reduced by the cytoplasmic swelling of endothelial cells; in some instances the endothelial swelling may appear to occlude the lumen. There may also be enlargement of the mesangium, which may accentuate the lobular pattern of the glomerulus. On immunofluorescence fibrin is frequently detected in capillary walls and mesangium. Immunoglobulin deposition is not prominent, although it has been reported in a number of cases (Fiaschi and Naccarato 1968, Vassalli et al. 1963). In the patient examined in the present study there was widespread fibrin deposition in glomerular capillary walls, associated with IgM and C_3 deposition to a lesser extent. This finding is in agreement with the report of Petrucco et al (1974) who described IgM and C_3 deposition in seven cases of toxaemia.

The aetiology of this condition is unknown. In some way intravascular coagulation is involved but the triggering mechanism

is not known. The finding of IgM and C_3 in the glomerular capillary walls raises the possibility that some immune stimulus has been released, and it is possible that this is feto-placental in origin. The majority of patients present after the thirtieth week of pregnancy, and it is known that at this time there is an increased plasma concentration of coagulation factors and an inhibition of fibrinolysis. It is possible, therefore, that this renders the kidney more susceptible to intravascular coagulation in response to a relatively minor stimulus such as antigen-antibody deposition. Intravascular coagulation is present in patients with pre-eclampsia as judged by increased fibrin degradation products (Bonner et al. 1971), low platelet counts (Brain et al. 1967), increased B-thromboglobulin (Redman et al. 1977) and increased fibrinogen turnover. The endothelial swelling may then interfere with the mesangial - JGA axis, so causing renin release and hypertension. This is speculative and does not explain many factors found in toxaemia such as the higher incidence in first pregnancies and the greater incidence in twin pregnancies, but at least it affords a working framework on which to base further study.

One patient in this series was found to have chronic pyelonephritis on biopsy. It is interesting to note that on immunofluorescence microscopy there was a granular deposition of IgG and complement (C_3) in glomerular capillary walls and also in the mesangium. It is difficult to know the significance of this deposition but there are several possibilities.

In chronic pyelonephritis there is repeated infection in the kidney and it is possible that this leads to antibody formation to the

infecting organism. There could then be slow release of antigen from bacteria with the subsequent deposition of complexes in the capillary walls. The fact that immune material also was located in the mesangium suggests that in this case the complexes were of a size for this pathway of removal. It is possible that the antigen is not bacterial but some material liberated by or altered by the infection, thus causing antibody formation. Obviously it would be of interest to study other cases of chronic pyelonephritis.

In five cases the renal lesion was tubular and there was no detectable glomerular disease. In these patients no immunofluorescence was detected and there was no reason to suspect any immunological component to the disease process. The negative findings are of value as they help to validate the findings in those disease states where there is good evidence that immune factors are of importance in the pathogenesis and/or progression of the disease. Similarly the negative findings in the three patients known to have normal renal function serve to act as 'controls' and add weight to the findings in patients with glomerulonephritis.

22. IMMUNOGLOBULIN DEPOSITION

Immunoglobulin deposition as detected by immunofluorescence occurs in a wide variety of glomerular diseases. In the majority of cases the immunoglobulin has been deposited as a result of some immunological reaction but other conditions, such as hypertension and systemic intravascular coagulation in which immunoglobulin deposition may occur, do not appear to be associated with any such process. However these cases are the exception and the fact that immunoglobulins are frequently detected in association with complement and fibrin support the concept that the majority of glomerular disease is immune mediated or has a significant immune component.

The detection of immunoglobulin or any other protein by immunofluorescence depends on several factors. The antiserum must be specific for the material being investigated, it must be adequately conjugated with fluorescein, it must be capable of combining with the antigen under laboratory conditions and it must respond to the exciting ultraviolet light. In addition the antigen must be present in amounts adequate for detection by immunofluorescence and its antigen sites must be exposed and capable of combining with the appropriate antibody. Thus in an immunofluorescence study there are many reasons why material may not be detected, and whilst a positive finding, if detected under properly controlled conditions, is of significance it is more difficult to place emphasis on a negative result. This stresses the need for very carefully controlled conditions when employing immunofluorescence to detect material in biopsy specimens and also underlines the need to monitor the specificity of the investigative materials continually.

In this study there did not appear to be any clear relationship of the type of immunoglobulin detected in the glomerulus to the underlying pathological condition. For instance, IgG, IgA and IgM were present in cases of proliferative, membranous and mesangiocapillary glomerulonephritis. As the exact function of these three immunoglobulins is unknown, this finding is not surprising. However, the pattern of distribution was closely related to the underlying disease and it was possible to differentiate between these three conditions on this basis.

The typical appearances have already been discussed in the relevant sections of this thesis. The fact that the immunoglobulin class can be so variable in different conditions rules out the possibility that the individual immunoglobulin is causally related in most conditions. Other factors such as antigen-antibody complex size, mesangial cell reactivity and extent of induced inflammatory response must be of considerable importance in determining the morphological changes which occur following an immune reaction.

Immunoglobulin G

Immunoglobulin G (IgG) has a molecular weight of approximately 160,000 and accounts for approximately 80% of plasma immunoglobulins. It is important in humoral defence against infectious disease. Following first exposure to an antigen there is a lag phase, where no antibody is detected in the plasma and this is followed by a phase when IgM is produced in increasing amounts. This phase lasts a few days and thereafter IgM production ceases and the concentration of this immunoglobulin falls in a manner similar to that of passively administered material. However, this is associated with the production

of increasing amounts of IgG over a period of about four days and there is evidence that after this period slow rates of IgG formation may continue for years. IgG, therefore, is not usually the first immunoglobulin to be produced in response to an antigen but it is produced in greater amounts and for a longer time than IgM. It is possibly for this reason that it is the most frequent antigen detected in patients with glomerular disease. However everybody is exposed to countless antigens throughout life and only a small number develop glomerulonephritis. In addition many patients appear to develop glomerulonephritis without any significant history of exposure to unusual antigens, such as infectious agents. Clearly there is much still unknown with respect to immunologically mediated glomerular disease.

IgG deposition seems to occur in the glomerulus in two basically different patterns. The first is a granular deposition in the capillary walls. The second is in a linear pattern along the basement membrane of glomerular capillaries. In the first it is considered that antigen-antibody complexes are deposited from the blood in the glomerular capillary walls by virtue of the size of the complex, the high blood flow rate per gram of tissue and the fact that the capillary is under considerable pressure when compared with other capillaries in the body. In the second type the IgG is directed against the basement membrane of the glomerular capillary and thus it becomes deposited on a fixed antigen, giving the typical linear immunofluorescence. These two patterns have been reproduced experimentally. Rabbits given a single intravenous injection of a large amount of purified plasma protein may develop a proliferative glomerulonephritis some 10 to 14 days later

(Hawn and Janeway 1947). It has been shown that the lesion develops at the time of removal of the antigen from the circulation due to soluble complex formation following antibody production (Germuth 1953). Dixon et al. (1958) showed that the antigen was deposited in the glomerular capillary walls at the time of antigen elimination, thus elegantly demonstrating the glomerular deposition of complexes. Further experimental evidence for immune complex mediated glomerulonephritis was offered by McClusky et al. (1962) who showed that intravenous soluble immune complexes prepared in vitro could produce glomerular lesions within 36 hours of injection.

Immunofluorescence studies have shown the complex deposition to have a granular or beaded appearance and thus it is reasonable to assume that the similar appearance seen in human renal biopsies is of a similar aetiology. The second pattern, that of a linear deposition, can be produced experimentally by the passive injection of heterologous antibodies against glomerular basement membrane or following immunisation with basement membrane preparations which stimulate the formation of autoantibodies. In both these circumstances immunoglobulin becomes deposited in a linear pattern along the glomerular basement membrane. In human disease the immune-complex type is by far the more common, the anti-GBM type being rare. This is confirmed in the present study where only one case of linear deposition has been noted.

Experimental studies have also given a clue as to why exposure to an antigen is not universally followed by an immune complex disease. Dixon et al. (1961) made a careful study of the relationship between antibody response and the development of lesions in rabbits given repeated injections of bovine serum albumin. He found that those

animals who produced no antibody and those animals who produced large amounts of antibody did not develop any signs of immune complex deposition. However those rabbits who produced poor antibody response, and were therefore presumed to have a prolonged soluble complex phase, eventually developed glomerular lesions. This has been confirmed by other workers (Andres et al. 1963, Germuth et al. 1967, and Pincus et al. 1968). It is thus possible that in the human situation the development of glomerulonephritis following exposure to an antigen, e.g. streptococcal antigen, is dependent upon the antibody response and the length of time that small soluble complexes are present. This could then explain why following an epidemic of streptococcal infection only a few people develop haematuria and proteinuria, although many have evidence of infection.

It is more difficult to explain the site of IgG deposition within the glomerulus. In patients with membranous glomerulonephritis the IgG is deposited in a subepithelial position and this is so for some of the deposits in post-streptococcal glomerulonephritis, although much of the deposit is in a subendothelial position in this condition. In lupus nephritis the IgG is in a subendothelial and subepithelial position while in mesangial IgG/IgA disease it is in the mesangium. If the only factor which determines glomerular deposition is that of size it is difficult to see why the IgG should be found in these different positions. It is possible that the site of deposition upon other factors such as the size, strength of charge on the antigen, or whether complement is activated at an early or late stage of the pathogenesis. However, it might just as easily be dependent upon as yet unidentified local factors such as mesangial

reactivity. The recent demonstration of receptors in the glomerulus which bind altered third component to complement (C_3b) is of interest and suggests another explanation for the glomerular localisation of complement-antigen-antibody complexes. Nonetheless IgG is rarely found solely in the mesangium and is most commonly present in the capillary walls alone. This is in contrast to IgA which, in spite of a similar molecular weight, is more commonly found in the mesangium. It may be that the IgG in the mesangium is altered in such a way that it is not detectable by immunofluorescence but this is unlikely as in mesangial IgG/IgA disease large amounts are found.

Material deposited in the glomerulus can be removed either by passing into the urine, into the systemic cirulation or into the mesangium. It is most likely that material is removed by all three routes. It certainly gains access to the mesangium and in resolving cases it is found frequently in such a site. It may be that this is a major route of removal of subendothelial material. In mice given ferritin material is found initially in a subendothelial position and with time there is gradual clearing from this position with accumulation in the mesangium. This may be the normal method of "clearing" the glomerulus and it may be only when this process is overwhelmed or inadequate that progressive disease results (Davison et al. 1974). The kinetics of removal of complexes are poorly understood and obviously require further detailed study.

Immunoglobulin M

Immunoglobulin M (IgM) differs from IgG particularly in respect to size; it has a molecular weight of approximately 900,000. It is most likely composed of five units each similar in structure to IgG.

The fact that the molecular weight is six times that of IgG is probably because the heavy chains of IgM are larger than the similar chains in the IgG molecule. IgM is capable of activating the complement cascade and appears to be particularly useful in enhancing the uptake of bacteria by phagocytes. The concentration of IgM in serum is approximately 10% of that of IgG; unlike IgG it is confined to the intravascular space.

IgM is detected in glomerular capillary walls and in the capillary walls and mesangium in a wide variety of glomerular diseases. It is only rarely found in the mesangium alone. IgM is normally produced as the first immunoglobulin in response to an antigen, and its production is usually limited to a short period of time after which the concentration falls in a manner similar to that of passively administered material. In addition, in the experimental situation the complexes which become deposited in the glomerular capillary walls are the soluble complexes which are found at a time of relative equivalence of antigen and antibody. The large complexes formed in antibody excess are not deposited in the kidney but are removed by the spleen. It is thus difficult to explain why IgM is found in the kidney in so many cases of glomerulonephritis. One would have expected that IgM would be produced for only a short period of time and that the complexes formed would be too large to be deposited in the kidney. If the IgM is deposited as part of a complex this may be additional evidence that in patients who develop glomerulonephritis there is some abnormality of the immune system.

It might be thought that IgM is involved because there is a deficit in IgG production. This seems highly unlikely because in

most instances IgM and IgG are present together and it is unusual to find IgM as the sole immunoglobulin. However, IgM may be produced to material liberated from the glomerulus following immune-complex deposition or complement activation. The IgM would therefore be present as a secondary phenomenon and not part of the initiating process. It would be equally possible that circulating IgM is deposited on material involved in an antigen-antibody reaction, such material being in normal circumstances in a physico-chemical state unsuitable for combining with IgM. At present it is not possible to state which, if any, of these propositions are valid. Little is known about the kinetics of IgM and its role in the pathogenesis of glomerular disease is unclear.

Immunoglobulin A

Immunoglobulin A (IgA) appears to exist in two major forms, circulating IgA and secretory IgA. Circulating IgA has a molecular weight of approximately 140,000, similar to that of IgG, and a concentration in the plasma approximately 25% that of IgG. Secretory IgA is present in mucous membranes and is associated with a 'secretory' or 'transport' piece which is a protein which is thought to help in the transfer of IgA from the submucosa to the lumen. The exact role of this secretory piece is not known and there is doubt whether it is necessary for the transfer of IgA. IgA appears to be secreted across membranes in the gastrointestinal tract and urinary tract and it is considered to offer local immunity against bacteria and viruses in these sites. The role of circulating IgA is unclear. It is considered by some that it gains access to the submucosa and by combining with the secretory piece is transferred through the mucosa.

However other workers are of the opinion that secretory IgA is produced by plasma cells in the submucosa and thus has no connection with circulating IgA. In support of this is the fact that in the submucosa there are considerable numbers of IgA containing cells; on the other hand IgA is of a size which would enable it to cross the capillary wall into the interstitial space. As yet the position is far from clear. IgA is not thought to fix complement by the classical pathway but the finding of IgA and complement together in many biopsies raises the possibility that it may activate complement by the alternate pathway.

IgA is found in a wide variety of glomerular diseases. It seems to be deposited in the mesangium more frequently and in larger amounts than either IgG or IgM. The reason for this is not clear. It is possible that immune complexes containing antigen and IgA are of a size to be preferentially taken straight into the mesangium, or it may be that such complexes are of such a physico-chemical constitution as to gain access to the mesangium more easily than other complexes. However IgA is an immunoglobulin of mucous membranes and it is certainly present in the urinary tract. It is interesting to note that hyaline casts present in a renal biopsy show strong immunofluorescence to IgA. It is possible that this is an entirely non-specific reaction but it is also possible that there is IgA in these casts and that the source is from the glomerulus. If this were the case the IgA may be secreted from the mesangium. The detection of IgA in the mesangium might therefore reflect no more than excessive production in response to some immune insult.

A major difficulty in the investigation of IgA in glomerular

disease is the difficulty of producing good specific antiserum. There is considerable species difference in the production of antibody to human IgA. The goat seems to be capable of producing a good titre of antibody, while the rabbit is not as effective. There is a need for a comparison of the different commercially available antisera to IgA to determine whether there is any significant difference in the biological activity of such materials. It is just possible that the different incidence of mesangial IgG/IgA disease, where the diagnosis is particularly dependent upon the finding of IgA in the mesangium, is because of different antiserum being employed (see V 9).

Immunoglobulin deposition is thus a frequent finding in the glomeruli of patients with glomerulonephritis. It is associated with complement and fibrin deposition and in the vast majority of patients indicates the immunological pathogenesis of the condition. However, why one class of immunoglobulin should be deposited predominately rather than another remains speculative and elucidation must await more detailed study.

Immunoglobulin E

During a small period of this study, antiserum to IgE became available. This was employed in the examination of 40 patients, but in only one patient was any positive immunofluorescence detected. This patient had a diffuse proliferative glomerulonephritis with a histological appearance suggesting a progressive lesion.

Gerber and Paranetto (1971a) described the deposition of IgE in the mesangial regions of four out of five patients with minimal lesion glomerulonephritis. Since then several other workers have failed to repeat this observation. In this study only three of

twenty patients with minimal lesion glomerulonephritis were examined with specific anti-IgE antiserum. None of these showed any evidence of IgE deposition.

Bennich and his co-workers (1974) studied 119 biopsies including eight of minimal lesion glomerulonephritis and only found two positive cases, both amyloidosis. Gerber and Paranetto (1971b) also described IgE deposition in the arterioles of patients with malignant hypertension, benign hypertension and some normal kidneys. This work has not been repeated and the most likely explanation is that there is some problem with the specificity of the antiserum.

Immunoglobulin D

Immunoglobulin D was first described by Rowe and Fahey (1965), but the exact role it plays in disease processes is unknown. Antinuclear antibodies of the IgD class have been found in systemic lupus erythematosus (Ritchie 1968, Watson et al. 1969) and IgD antibodies have been detected against thyroglobulin, insulin, penicillin and milk proteins.

In this study 76 biopsies were examined with anti-IgD serum and no specific immunofluorescence was detected in any of them. This is in contract to the results of Katz and Pruzanski (1975) who found it in eight of 263 consecutive biopsies and in all cases the IgD was associated with IgG and Beta 1C deposition while, in addition, IgM was present in four instances. It is difficult to compare two different studies but Katz and Pruzanski (1975) found positive deposition in four of thirteen cases of membranous glomerulonephritis. Assuming this incidence to be real we would have expected to have seen some deposition in membranous glomerulonephritis but I have found

no positive immunofluorescence in the nine cases examined. Glomerular localisation of IgD has also been reported by Kantor et al. (1970) who found focal deposits in two patients with proliferative glomerulonephritis and in one renal allograft. Tarantino et al. (1974) found IgD deposition in amyloidosis and diabetes mellitus. Unfortunately in the present study no patients with amyloidosis or diabetes mellitus has been examined with IgD antiserum.

23. COMPLEMENT

Complement is a series of plasma proteins which are activated in a cascade system rather like coagulation. In the "classical" system the first component (or components) of complement (C_1) is activated by an immune complex. The active component then forms a complex with the fourth component of complement (C_4) and this in turn activates C_2, C_3, and then C_5 and C_9. The result of this action is that there is lysis of cells, bacteria and viruses. In addition various fragments are split from the inactive components and these fragments have actions such as attraction of polymorphs, the release of inflammatory factors and the enhancement of the activation of the coagulation system. There is a second method of complement activation, the so-called 'alternate' pathway, in which the third component of complement is activated without involvement of the earlier components. It is considered that there is a factor which will convert inactive C_3 to an active form which then causes activation of the components from C_5 to C_9. This factor, thought to be an immunoglobulin, has been termed the nephritic factor (C_3NeF) since it is found in the serum of patients with mesangiocapillary glomerulonephritis and hypocomplementaemia. Complement is thus an important factor in producing the inflammatory response which follows antigen-antibody reactions.

Complement deposition can be found in a wide variety of renal diseases and it is, as expected, frequently associated with immunoglobulin and/or fibrin deposition. It is found in those conditions in which there is good evidence of an immunologically mediated injury such as proliferative, membranous and mesangiocapillary

glomerulonephritis. In such conditions it is reasonable to suppose that the complement, C_3 or C_4, has been deposited in close association with antigen and immunoglobulins. However in many instances of these forms of glomerulonephritis no complement can be detected, e.g. C_3 was present in only five of seventeen cases of membranous glomerulonephritis and 38 of 83 cases of proliferative glomerulonephritis. The lack of immunofluorescence may be explained by supposing that the amount of complement was too small to be detected or that the antigenic sites were not available to combine with the appropriate antiserum. However it is possible that complement is involved only in the early stages of an inflammatory reaction and that its presence is only transient. To support this latter idea is the fact that one of the important actions of complement is the attraction of polymorphs with the subsequent liberation of 'toxic' factors. It is known that the polymorph infiltration in experimental glomerulonephritis is an early and transient event although the consequences of such attraction may be evident for a considerable period of time. In addition in membranous glomerulonephritis complement (C_3 or C_4) is only rarely found after twelve months from the onset of clinical symptoms (IV 3) but this negative finding does not imply that complement has not been a significant factor in the pathogenesis of the disease.

Of considerable interest in the understanding of immune complex localisation is the recent detection of complement (C_3) receptor sites in the glomerulus (Gelfand et al. 1975). This finding suggests a specific mechanism for the deposition of immune complexes in immune-complex-mediated glomerular injury. Gelfand and his co-workers

have demonstrated receptors in the human renal glomerulus which selectively bind the activated third component of complement (C_3b) (Gelfand, Frank and Green 1975), and it is possible that activated complement complexed with antigen and antibody becomes bound to such receptors. Further work from this and other groups show that in human renal lesions associated with in vivo deposition of C_3 there is a loss of these receptor sites as demonstrated by reduced or absent in vitro binding of C_3b. (Gelfand et al. 1976, Burkholder et al. 1977). In view of the technique for detecting these receptors there has been considerable difficulty in localising their exact position in the glomerulus. Originally it was considered that the receptors were located in endothelial cells (Gelfand et al. 1975a) but it has also been suggested that they are in epithelial cells (Gelfand et al. 1976, Burkholder et al. 1977) and mesangial cells (Sobel et al. 1976). The significance of these findings is not known but it offers an attractive hypothesis to explain the presence of antigen, antibody and complement in patients with immune mediated glomerulonephritis.

C_3 was found in one case of minimal lesion glomerulonephritis. This is unusual but may reflect the fact that in a few patients with this condition there is a small amount of IgM deposition, and indeed this was the case in three of the twenty-four biopsies studied in this series. However this is similar to other reported series and it is also known that IgM is more effective in 'fixing' complement than any other class of immunoglobulin.

It is also of interest to note that C_3 was detected in two patients with acute tubular necrosis, two with disseminated

intravascular coagulation and one with hypertension. The two patients with acute tubular necrosis were studied late in their illness and it is just possible that some glomerular material was liberated at the initial insult and that this then initiated an immune reaction. This is supported by the fact that IgM was also present in these two cases. However, it may represent an immunological reaction to the agent causing the acute renal failure and therefore may be a mild immune complex reaction insufficient to cause any other morphological change. The same explanation may be possible in the two cases of disseminated intravascular coagulation but the patient with essential hypertension cannot be explained in this way.

Antiserum to C_4 was available only towards the end of the study and the deposition of this material closely followed that of C_3, indicating a classical pathway of complement activation. It was present in 22 of 77 cases examined. It was present in two of four cases of mesangiocapillary glomerulonephritis but unfortunately these two cases were investigated before it became possible to estimate C_3NeF and so it is not possible to state that C_4 deposition occurs in hypocomplementaemic glomerulonephritis. In membranous glomerulonephritis there was only one patient with small amounts of C_4 deposition who did not have any C_3 identified but in the other cases C_3 and C_4 existed together indicating a classical pathway activation.

In a study of complement excretion in the urine (Cumming et al 1976) there was a close association between the presence of complement in the urine and glomerular complement deposition. This would therefore seem to provide a method of detecting immunologically

mediated glomerular disease and clearly this is worthy of further investigation.

Complement identification in the glomeruli of patients with glomerulonephritis is thus further evidence of the immune basis for such conditions. It is not observed as frequently as immunoglobulins but this may be only a reflection of the fact that complement is involved early in immunological inflammation and in a transient manner.

24. FIBRIN

Glomerular fibrin deposition is common in a wide variety of diseases. Indeed in this study it was the most common finding on immunofluorescence microscopy. It was usual to find fibrin deposited in capillary walls, but occasionally the deposition was in walls and mesangium. Only rarely was fibrin detected in mesangium alone.

There is little doubt that immunofluorescence has been of prime importance in demonstrating the role of coagulation in renal disease. Prior to the introduction of immunofluorescence the detection of fibrin was solely dependent upon histochemical techniques. There is a poor relationship between histological stains and the immunofluorescence detection of fibrin and this is hardly surprising in view of the dynamic nature of fibrin deposition. False positive histological results occur in conditions where there is mesangial accumulation of certain materials, as in diabetic glomerulosclerosis and amyloidosis, and false negative results tend to occur in those situations where only a small amount of fibrin is deposited. Once fibrin has been formed from fibrinogen, it undergoes certain changes due to exposure to factor XIII, the fibrin stabilising factor, and subsequent exposure to fibrinolytic enzymes probably produces steric changes which will affect the affinity for histological stains. Fibrin antiserum, however, will detect fibrinogen, fibrin and certain of the fibrin degradation products and therefore more accurately reflects the presence of fibrin.

The relative lack of fibrin within the mesangium is difficult to explain. It is possible, however, that the fibrin degradation products are small enough to pass through into the urinary space and are washed

out in the urine rather than being removed through the mesangium. It may well be that fibrin is detected within the mesangium only when it is present in the capillary walls in large amounts. There is a close correlation between urinary fibrin degradation product excretion and glomerular fibrin deposition (Davison et al. 1973) and thus the serial measurement of urinary F.D.P. provides a good method of following the state of intrarenal fibrin deposition and therefore the natural history of response to therapy of various forms of glomerulonephritis.

Fibrin deposition within the glomerulus is probably usually consequent upon an immunologically mediated injury. It is important in the pathogenesis of glomerulonephritis and in experimental models it has been shown that defibrination (Thomson et al. 1975, Naish et al. 1972) or anticoagulation prior to exposure to immune complexes (Vassalli and McClusky 1964, Halpen et al. 1965) prevents or significantly attenuates glomerular damage. In the human situation it is most likely that the immune complex becomes deposited in the glomerular capillary wall and activates the complement cascade. There is subsequent polymorph attraction with the liberation of kinins and the activation of the coagulation system. The subsequent events will then depend to a large extent on the ability of the kidney to recover from such an insult. If the insult is single and short-lived, recovery is to be expected when the consequences of the insults have been naturally resolved. However, if the precipitating factors are persistent, a progressive disease is likely to develop, particularly when the amount of fibrin being deposited is in excess of that that may be removed by lysis and/or the mesangial system.

In the present study this is supported by the finding that in progressive conditions there are frequently large amounts of fibrin in glomerular capillary walls and the mesangium. Intravascular coagulation has recently received much attention. It can be suggested that in most cases of glomerulonephritis there is localised intravascular coagulation consequent on the immune injury. However, in this study a few cases have been examined in which the renal disease was part of a more widespread or disseminated intravascular coagulation, (see Disseminated Intravascular Coagulation V, 11).

25. CLINICO-PATHOLOGICAL CORRELATIONS

Primary glomerular disease may present in only a few ways. The patient may be asymptomatic and only be detected at some routine medical examination or he may present with either the syndromes of acute nephritis, recurrent haematuria, nephrotic syndrome, acute renal failure or chronic renal failure. However, when the glomerulus is involved as part of the systemic disease it is frequently manifestations other than those due to renal involvement that produce symptoms. Glomerular disease may make itself manifest by haematuria, proteinuria, hypertension and/or diminished renal function. It is not surprising, therefore, that there is a poor relationship between the clinical presentation and the final diagnosis in a patient with primary glomerular disease (Table 26, page 207).

In view of the limited clinical manifestations of glomerular disease many conditions may present in the same way. The only good correlations appear to be that rapidly progressive glomerulonephritis presents as acute renal failure and minimal lesion glomerulonephritis presents as nephrotic syndrome. The converse, however, is not true. Patients with nephrotic syndrome may have a wide variety of glomerular disease and similarly there may be many causes for a presentation of acute renal failure. Many conditions may not become manifest until late in the natural history of the disease. This is supported by the fact that a large number of patients in our study were asymptomatic and were only detected at routine medical examination. If a patient has a mild glomerular disease which results in proteinuria of less than 5 grams per 24 hours it is unlikely that the nephrotic syndrome will develop. In addition many patients with glomerular disease are

not hypertensive and so if such a patient were to have a progressive condition they may not be identified until chronic renal failure becomes obvious. In the present study relatively few people were included with a presentation of chronic renal failure. The reason for this is that many such patients have small contracted kidneys and as such are not suitable for renal biopsy.

The relationship between functional abnormalities and structural changes has been studied by many authors since the first classification devised by Bright (1827). There appears to be little correlation between glomerular disease and renal function. Several workers have demonstrated that impaired renal function is more closely related to changes in the tubules and interstitium irrespective of the severity of the glomerular lesions, (Risdon et al. 1968, Schainuck et al. 1970). The reasons for this are not clear but it may well be that the lesions seen on light microscopy in proliferative and other forms of glomerulonephritis have little effect on glomerular filtration rate. Significant changes in function would therefore depend upon nephron loss due to complete glomerular hyalinisation and its subsequent effect on the post-glomerular capillary network. This may wellexplain the relationship between rapidly progressive glomerulonephritis and acute renal failure. In rapidly progressive glomerulonephritis there is considerable glomerular destruction and secondary tubular effects are common. In most other glomerular diseases the tubular effects are relatively limited until late in the natural history of the disease, and in such instances are usually associated with progressive glomerular loss.

The relationship between the clinical presentation and histological

diagnosis (Table 27, page 209) is extremely poor. The reason for this is not difficult to understand in view of the limited ways in which glomerular disease may present and the large number of conditions which may affect the glomerulus. The histological diagnosis and final diagnosis (Table 28, page 211) are much better correlated and this emphasizes the importance of renal biopsy in patients with renal disease. In certain conditions there are characteristic biopsy changes but in most patients account has to be taken of the clinical presentation, the biochemical findings and the biopsy findings before a final diagnsis can be reached. For instance, membranous glomerulonephritis may be seen in systemic lupus erythematosus, amyloidosis and idiopathic membranous glomerulonephritis. Immunofluorescence microscopy may differentiate between lupus erythematosus and amyloidosis but electron microscopy may well be necessary to make a diagnosis of idiopathic membranous glomerulonephritis. Even when this diagnosis is reached it is not possible to state whether the changes are due to an underlying malignancy, malaria or a drug such as penicillamine.

The classification of glomerulonephritis can be made on the basis of clinical presentation or histological findings. Both these have limitations and will not give an accurate diagnosis. In reaching a final diagnosis in any patient it is clearly necessary to take account of the presentation, the biochemical and serological investigations and the biopsy findings.

26. MESANGIUM

The mesangium consists of mesangial cells and mesangial matrix and it is situated in the junction between glomerular capillary loops. The term mesangium was first used by Zimmerman (1933) who described it as a connective tissue stalk extending from the glomerular hilum to the peripheral capillary lobules providing support for the capillaries. The mesangial cells have been variously termed third cells, deep cells, centri-lobular cells or stalk cells but there is now general agreement on the term mesangial cell. There is considerable interest in the function of the mesangium and many believe it to be phagocytic and an extension of the reticulo-endothelial system.

Latta and Maunsbach (1962) demonstrated that the rat mesangium would take up rapidly particles of thorium dioxide following intravenous injection. It appeared that the thorium particles entered the mesangium and passed through the matrix to the hilum of the glomerulus and then into the granular cells of the juxtaglomerular apparatus. Similar results were obtained by Farquhar and Palade (1962) who found ferritin within the mesangial matrix within five to ten minutes of an intravenous injection, demonstrating the rapidity with which material enters the normal mesangium. These results have been obtained by other workers using colloidal iron, human globulin and colloidal carbon. Injected material appears to localise first in the sub-endothelial position, then moving towards the mesangium and subsequently to the juxtaglomerular apparatus. It has been concluded, therefore, that the mesangium is phagocytic and its purpose is to clear material which may be deposited in the subendothelial position during the process of glomerular filtration.

In experimental animals it has been shown that complexes become deposited in the mesangium, and that this is associated with cell hyperplasia and increase in mesangial matrix (Suzuki et al. 1963). It is possible that the proliferation which occurs in many forms of glomerulonephritis is a mesangial response to immune complexes.

It is possible that the immune complexes do not accumulate in the mesangium purely as a result of filtration. Recently Gelfand and his co-workers (1975) have demonstrated receptors in the human renal glomerulus which selectively bind activated third component of complement. This suggests the possibility that immune complexes containing activated complement are selectively bound in the mesangium and possibly also the glomerular capillary wall. This is supported by the finding that there was a loss of receptor sites in the renal biopsies of the patients with lesions associated with in vivo deposition of complement (C_3).

Regardless of the method of deposition of immune complexes or inflammatory products in the mesangium it would appear that the mesangium affords a route whereby such substances may be eliminated. In glomerular disease the progression of a given lesion may depend upon the balance between deposition of such material and effective removal. This is supported in the present series by the finding that deposition of immunoglobulins or fibrin in large amounts in the mesangium, or in the capillary walls and mesangium, is associated with progressive disease. Recovery from glomerulonephritis may therefore be to a large extent dependent upon the ability of the mesangium to clear deposited material and restore the capillary wall and lumen to normal.

There are still many unanswered questions with respect to the function of the mesangial matrix and mesangial cell. It is interesting to note that the mesangial reaction is so variable in glomerulonephritis and yet there are certain consistent patterns in certain disease states. For instance in persistent hypocomplementaemic glomerulonephritis the lesion is of the mesangiocapillary type. Similarly in mesangial IgG/IgA disease, although the mesangium contains large amounts of IgA and IgG associated with some complement (C_3), there is little mesangial proliferation and only a modest increase in mesangial matrix. There is a possibility that a given stimulus evokes a particular response and therefore a particular histological appearance. However, it is just as possible that an individual's mesangium is only capable of reacting in a given way and that the histological appearance is more dependent on the patient than the initiating event.

VI SUMMARY OF FINDINGS

1. Three hundred biopsies were performed in 267 patients. In 42 patients a biopsy had been performed prior to the one in this study. In 28 patients a repeat biopsy was performed during this study. In 162 patients the biopsy was undertaken to establish a diagnosis. In 19 patients there was a suspected systemic disease and in 25 there was a condition known to affect the kidneys. Thirty-seven biopsies were undertaken to learn more about the natural history of the disease process and in 33 cases the biopsies were performed to determine the effect of therapy on the histology. Biopsies were obtained from 22 transplant patients and also from two patients, with normal kidneys, who were undergoing urological surgery.

2. Diffuse proliferative glomerulonephritis was the most common condition encountered being present in 80 biopsies from 73 patients. The most common clinical presentation was nephrotic syndrome being the initial symptom in 19 cases. Of the remainder the presentation was as acute nephritis in 14, as asymptomatic proteinuria in 14 and as recurrent haematuria in 13 patients.

The most common immunofluorescence finding was a granular deposition of fibrin/fibrinogen in glomerular capillary walls. This occurred in 43 patients. In 32 patients there was deposition of IgM and complement (C_3) in a granular fashion in capillary walls. In 30 cases IgG was present and in 20 cases IgA was present. Immunofluorescence was also relatively frequently found in capillary walls and the mesangium but was only rarely found in the mesangium without

any being present in the capillary walls.

At follow-up examination the following features appeared to be associated with a poor prognosis, the presence of hypertension as initial presentation, the older the patient, the presence of a progressive lesion on histological examination, and the presence of immunoglobulins and/or fibrin in large amounts in glomerular capillary walls and the mesangium.

3. Rapidly progressive (crescentic) glomerulonephritis was present in eight biopsies from five patients, (3 females and 2 males). The average age at presentation was 56 with a range from 34 to 69 years. In all patients the presentation was as acute renal failure. Respiratory symptoms were common and hypertension rare.

On light microscopy there were large circumferential crescents in the majority of glomeruli. On immunofluorescence examination fibrin/fibrinogen was detected within the crescents. Only small amounts of IgG, IgM and complement (C_3) were detected in the glomerular capillary walls. In no case was there linear deposition of immunoglobulins or complement.

The prognosis in this group was particularly poor with improvement in renal function occurring in only one of the five patients.

4. Seventeen biopsies were obtained from sixteen patients with membranous glomerulonephritis. There was a male preponderance in this group (13 males, 3 females). The mean age at onset of symptoms was 50 years but there was a wide scatter from 22 to 74 years. The most common mode of presentation was as nephrotic syndrome and membranous glomerulonephritis accounted for 32 per cent of adults presenting with nephrotic syndrome. Hypertension was present in half of the

patient but haematuria was relatively rare, being present in only three.

On light microscopy there was a diffuse uniform increase in basement membrane thickness. On immunofluorescence examination the most common finding was a granular deposition of IgG along all glomerular capillary walls. In some instances this was associated with IgA or IgM deposition. Complement and fibrin deposition was relatively rare. There was no immunofluorescence visible within the mesangium. The immunofluorescence were related to the time between the onset of symptoms and the biopsy. Little immunofluorescence was visible prior to eight months and none was present after 72 months.

At follow-up examination only two patients had shown any improvement in their renal function, seven remained stable and seven showed a steady deterioration.

5. Mesangiocapillary glomerulonephritis was present in 14 biopsies from 13 patients. There were nine males and four females in this group and their ages ranged from 7 to 61 years with a mean of 31 years. Proteinuria, haematuria, impaired renal function and hypertension were common in this group.

There were seven patients with the subendothelial deposit type of glomerulonephritis and six with the dense deposit type disease. The immunofluorescence did not bear any relationship to the ultrastructural type. The immunofluorescence had a characteristic granular deposition in the peripheral walls of glomerular capillaries. IgG, complement (C_3) and fibrin/fibrinogen all occurred with equal frequency. IgM and IgA deposits were less frequent. The immunofluorescence did not have any relationship to the time since onset of symptoms or the outcome of the

disease.

At follow-up examination only one patient has shown any improvement.

Two biopsies were obtained from two patients, both females, with partial lipodystrophy. On light microscopy both had the dense deposit type of mesangiocapillary glomerulonephritis and the immunofluorescence findings were the same as other patients with this histological diagnosis.

6. Focal proliferative glomerulonephritis was present in 12 biopsies from 11 patients. These biopsies were obtained from eight male and three female patients with a mean age of 29 but a wide age range between 3 and 71 years. The clinical presentation was extremely variable and from the clinical history there was not any clue as to the underlying aetiology.

The most common immunofluorescence finding was a granular deposition of IgM within glomerular capillary walls. This was associated with some deposition of IgG, IgA, complement and fibrin/fibrinogen. The immunofluorescence distribution appeared to be fairly uniform throughout glomeruli except one case where there appeared to be some focal accentuation.

The prognosis was not good with three patients showing a progressive deterioration to renal function. Neither the mode of presentation nor the immunofluorescence findings provided any indication of the prognosis.

7. Only two patients with mesangial IgG/IgA disease were studied. Both were young males, one presenting with asymptomatic proteinuria and the other with recurrent haematuria. Both were hypertensive but only one had significant proteinuria.

On light microscopy there was a moderate increase in mesangial

matrix. On immunofluorescence microscopy there was a marked granular deposition of IgG, IgA and complement (C_3) within the mesangium, but there was very little immunofluorescence within glomerular capillary walls.

One patient has been lost to follow-up but the second continues with good renal function but with the syndrome of recurrent haematuria.

8. Twenty-four biopsies were obtained from twenty patients with minimal lesion glomerulonephritis. The mean age was 15 years but there was a wide range from 1 to 70 years although only two patients were aged over 20 years. All presented with nephrotic syndrome and proteinuria varied between 2.8 and 20 grams per 24 hours. No patient had haematuria or hypertension.

On light microscopy the only changes were of a minor proliferation of mesangial cells. On immunofluorescence examination eight biopsies showed a small amount of fibrin/fibrinogen within glomerular capillary walls. In one case some IgM was also present. Three biopsies were examined for deposition of IgE but all were negative.

At follow-up examination all patients had normal renal function and were in remission. All had been treated with Prednisone but in seven cases a combination of Prednisone and Cyclophosphamide was required. The prognosis in this condition is good and none of the patients has progressed to renal failure.

9. Focal glomerulosclerosis was present in five patients (3 males, 2 females). The initial presentation was as nephrotic syndrome in two cases, asymptomatic proteinuria in two cases and as hypertension in the remaining case. On examination 24 hour protein excretion ranged between 2 and 4 grams, four patients had haematuria and four

patients were hypertensive.

On light microscopy many glomeruli were completely hyalinised. In the remainder there were irregular enlarged mesangial regions which in some cases were sufficient to obliterate capillary lumina. On immunofluorescence two patients were negative but in the remaining three patients there was granular deposition of IgG, IgM, fibrin and complement.

At follow-up examination only one patient returned to normal and the remaining four patients still have significant proteinuria and hypertension.

10. Twelve biopsies were carried out in eleven patients with Henoch-Schonlein purpura (7 males, 4 females). The age range was between 5 and 63 years but there were only three patients older than 17 years. There was a typical clinical presentation with haematuria, purpura, joint pains and abdominal pains. No patient was hypertensive. Proteinuria was variable being insignificant in five cases and ranging from 0.4 grams to 7 grams per 24 hours in the remaining six. The creatinine clearance was diminished in four patients.

On light microscopy there was a proliferative glomerulonephritis. In four patients this had a focal accentuation. The most common immunofluorescence finding was of IgG, IgA and fibrin/fibrinogen in glomerular capillary walls. In only two patients was any IgM present. Fibrin was present in crescents in two instances.

At follow-up examination the illness regressed completely in seven patients. In two patients renal function progressively deteriorated and these patients subsequently required intermittent haemodialysis.

Those features indicating a poor prognosis were a diminished renal function at initial presentation, an elevation of the ESR, crescents on light microscopy and a significant polymorph infiltration on light microscopy.

11. Biopsies were obtained from nine patients with acute tubular necrosis. All patients had acute oliguric renal failure consequent upon some specific incident.

Immunofluorescence examination revealed that the most common finding was a diffuse staining of the oedematous inter-tubular regions with fibrin/fibrinogen. In only four cases was fibrin present within glomeruli. In two patients there was a weak granular deposition of IgM and C_3 within glomerular capillary walls.

12. Nine biopsies were performed in seven patients with disseminated intravascular coagulation. All patients presented with a short preceding illness. Disseminated intravascular coagulation was diagnosed on the basis of thrombocytopenia, anaemia, raised plasma haemoglobin, reduced plasma haptoglobin and a typical blood film appearance. In two patients renal function was not seriously impaired but in three there was acute renal failure requiring treatment by intermittent haemodialysis.

The immunofluorescence findings revealed a diffuse granular deposition of fibrin in glomerular capillary walls in five of the seven patients. There was an associated fibrin deposition within arterioles in four patients.

In this group of seven patients there were four deaths and three patients recovered normal renal function.

13. Ten biopsies were obtained from ten patients with systemic lupus

erythematosus. All patients in this group were female and their age at initial presentation varied between 4 and 46 years (an average of 28 years). The initial presenting features included a typical skin rash, arthropathy, Raynaud's phenomenon, nephrotic syndrome and purpura. All patients except one had haematuria and proteinuria varying between 0.8 and 10.7 grams per 24 hours was present in eight of the ten patients. Seven were significantly hypertensive.

On light microscopy all had a proliferative glomerulonephritis and the typical so-called wire-loop lesion was present in only three cases. On immunofluorescence the most common finding was of fibrin deposition within the glomerular capillary walls. This was most frequently associated with IgG deposition although IgM was present in three cases. Complement was not a significant finding except in one patient. Mesangial deposition was uncommon.

At follow-up six patients showed no evidence of declining renal function. Three patients showed a steady deterioration and one died from acute renal failure.

14. Thirteen biopsies were carried out in twelve patients with polyarteritis. The average age of the patients was 55 years (range 35 to 65 years). In six patients the initial clinical presentation was with respiratory symptoms and two patients presented with acute renal failure. Proteinuria was not a common finding and hypertension was rare.

On light microscopy all patients had microscopic polyarteritis and there was no patient with polyarteritis nodosa. On immunofluorescence microscopy the most common finding was a granular deposition of fibrin within glomerular capillary walls. In only four

patients was IgG present. There was little immunofluorescence in mesangial regions. In arterioles complement (C_3) was visible in one case and in one further case IgM was present in arteriolar walls.

At follow-up examination six patients remained essentially unchanged. All except one were treated with steroids. Three patients died and one progressed to intermittent haemodialysis.

15. Two patients with scleroderma were biopsied. On immunofluorescence microscopy the thickened intima of small arterioles and interlobular arteries contained deposits of IgM and complement (C_3).

16. Renal biopsy was carried out in 21 patients with hypertension. Ten of these patients had malignant hypertension. On immunofluorescence microscopy no specific immunofluorescence was detected in the eleven patients with essential hypertension. In those with malignant hypertension five patients had a minor deposition of fibrin in a granular fashion in glomerular capillary walls. This was associated with small amounts of IgG in two patients and small amounts of IgM in a further two patients. The most striking feature on immunofluorescence was an autofluorescence of the re-duplicated and split elastic lamina in arterioles.

17. Twenty-two biopsies have been examined from transplanted kidneys in eighteen patients. In ten instances the biopsy was obtained because of primary non-function, eight were obtained during rejection episodes and three were obtained at post mortem.

In the biopsies obtained during the investigation of primary non-function the most common finding was a diffuse scattering of IgM containing cells in the interstitium. In biopsies undertaken during a rejection episode the most common finding was of IgM, complement (C_3)

and fibrin/fibrinogen within small arterioles. In addition there was occasionally some IgM containing cells in the interstitium and in three cases there was a significant amount of fibrin deposited in a granular fashion in glomerular capillary walls. Only one case showed extensive deposition of IgG, IgA, IgM and C_3 in glomerular capillary walls.

18. Thirteen biopsies were performed in thirteen patients with diabetes mellitus. On immunofluorescence microscopy there was a small amount of fibrin visible in the capillary walls of six of the thirteen biopsies. In one case the fibrin was present in a peripheral capillary wall and appeared to correspond to a fibrin cap. IgM was present in the capillary walls of three biopsies. In one case IgM was present in the intima of arterioles. The immunofluorescence findings did not appear to bear any relationship to the time of onset of diabetes, whether the patient was receiving insulin or oral hypoglycaemics, or whether he had proteinuria, hypertension or diminished renal function.

19. Six biopsies were carried out in six patients with amyloidosis. In three patients the amyloidosis was primary whilst in three patients it was secondary. All patients exhibited proteinuria but none showed haematuria. None were hypertensive but all had diminished renal function at initial presentation.

On immunofluorescence microscopy IgG was present in the expanded mesangial regions of two cases and one of these cases had in addition IgG within arteriolar walls. In one further case a small amount of IgG was present in glomerular capillary walls. In the three cases with primary amyloidosis no specific immunofluorescence was detected.

20. A patient with a malignancy associated nephrotic syndrome was studied. On initial biopsy there were large deposits of IgG and Ig^M in the glomerular capillary walls. In some places the deposits appeared large enough to occlude the capillary lumen. Five months after hysterectomy for choriocarcinoma a repeat biopsy was performed. Immunofluorescence at this time revealed only a weak deposition of IgM associated with a small amount of complement (C_3) in glomerular capillary walls. The large deposits visible on the initial biopsy had resolved completely.

21. Renal biopsies were performed in five patients with predominently tubular disease and two patients with no known renal disease. In all these instances immunofluorescence was negative.

22. IgG was frequently deposited in proliferative glomerulonephritis, membranous glomerulonephritis, mesangiocapillary glomerulonephritis and systemic lupus erythematosus. It was most commonly found in glomerular capillary walls or in the walls and mesangial regions and seldom solely in the mesangium. In all cases the deposition was granular although in some cases the small granular deposits appeared confluent and gave the appearance of short linear deposits. In many instances the pattern of deposition was characteristic of a specific condition such as the uniform granular deposition in membranous glomerulonephritis and the deposition in peripheral walls of glomerular capillaries in mesangiocapillary glomerulonephritis.

23. IgA was most commonly detected in the mesangium or capillary walls and mesangium. It was relatively infrequently found in the capillary walls alone. In all cases the deposition was granular. Tubular casts frequently gave bright immunofluorescence to IgA.

24. IgM was most commonly noted with a granular distribution in glomerular capillary walls. It was most common in cases of proliferative glomerulonephritis but was also found in membranous glomerulonephritis, mesangiocapillary glomerulonephritis and focal proliferative glomerulonephritis. In transplant patients undergoing rejection IgM was occasionally found in glomerular capillary walls.

25. IgE was only detected in one biopsy, that of a patient with diffuse proliferative glomerulonephritis.

26. No specific immunofluorescence to IgD was detected in the 76 biopsies examined.

27. Complement (C_3) was detected in 75 of 300 biopsies. It was most frequently detected with a granular distribution in glomerular capillary walls. In only 11 cases was it detected solely in the mesangium. It was most commonly found in proliferative glomerulonephritis.

28. Complement (C_4) was detected in 11 of 77 cases examined. It was most commonly present in membranous glomerulonephritis and mesangiocapillary glomerulonephritis.

29. Fibrin was detected in 126 of 300 biopsies. It was most frequently detected in glomerular capillary walls and in only four instances was it found solely within mesangial regions. It was most common in proliferative glomerulonephritis but was also noted frequently in transplant biopsies and was present in all cases of disseminated intravascular coagulation.

Immunofluorescence microscopy appeared to be the most sensitive method of detecting fibrin deposition within renal biopsies.

30. There is no clear relationship between clinical presentation and final diagnosis.

31. There is no relationship between clinical presentation and histological diagnosis.

32. There is a reasonable correlation between histological diagnosis and final diagnosis although in such conditions as Henoch-Schonlein disease there may be a wide variety of histological appearances.

33. The mesangial cell appears to play an important part in patients with proliferative glomerulonephritis. If on immunofluorescence microscopy, large amounts of immunoglobulins or fibrin are detected within the glomerular capillary walls and mesangial regions the prognosis is less satisfactory than when the immunofluorescence is confined to the capillary walls or is absent.

VII ACKNOWLEDGEMENTS

I am extremely indebted to many people for their kind help, stimulation and encouragement with this study. To Professor J. S. Robson I owe a particular debt for kindling my interest in renal disease and particularly glomerulonephritis. Without his stimulation this study would not have commenced and without his continued encouragement it would not have been completed. In addition to Dr. Mary K. MacDonald and Dr. David Thomson I owe more than words can express. Throughout this study, and since, they have provided a continued academic dialogue from which much of the original study has grown and from which many ideas have germinated and come to fruition. Their untiring help and encouragement has been much appreciated.

This study was undertaken in the Department of Pathology at the University of Edinburgh under the guidance of Dr. Mary K. MacDonald. It is with real pleasure that I acknowledge my thanks to Professor Emeritus G. L. Montgomery and Professor A. R. Currie for extending to me the facilities of that department. During 1972 and 1973, when the department was grossly overstretched, my work was enabled to proceed without interruption and for this I am most grateful.

Technical help with immunofluorescence and light microscopy has most willingly been given by Mr. J. K. Rae and I wish to record my thanks to him for his hard work and excellent technical expertise. I am also obliged to Mr. W. Donaldson and Mr. I. Banks for technical help with respect to electron microscopy and the quality of the electron micrographs in this thesis is a testimony to their ability.

This study has involved many clinicians and I am most grateful for their ready willingness to supply material and clinical details. I would particularly like to thank Dr. John Anderton, Professor J. Crofton, Sir John Halliday Croom, Dr. Andrew Doig, Dr. Leslie Duncan, Dr. A. J. Kay, Dr. Anne Lambie, Dr. John Munro, Dr. Michael Oliver, Dr. R. Robertson, Dr. W. Uttley, and Sir Michael Woodruff, who have all allowed me access to case notes to obtain follow-up details of patients. Without this help it would not have been possible to obtain the clinico-pathological correlations which form an important part of this thesis.

The urinary fibrin/fibrinogen estimations were the work of Dr. Tony Clarkson who initiated much of the present interest in the involvement of coagulation in glomerulonephritis. I am grateful for having worked with him and for having so many discussions on the pathogenesis and natural history of glomerulonephritis.

I am obliged to the Scottish Home and Health Department for the financial support of this work and for their continued interest in the results of the study.

The photographic illustrations are the work of Mr. J. Paul of the Medical Illustration Department of Edinburgh University and Mr. D. Howard of the Department of Medical Illustration of St. James's University Hospital, Leeds.

I am most grateful to Mrs. Catherine Kidson for secretarial assistance in preparing this manuscript, which by now she must know almost by heart.

Finally my most sincere thanks and apologies must go to my family for the uncomplaining way in which they have tolerated the long

gestation of this thesis. Their home has long been disrupted with reprints, proofs, results and analyses, and their weekends interrupted by my working. To Marion, Andrew, Pamela and Iain I owe most not only for their tolerance and understanding, but for their encouragement and interest in my study.

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